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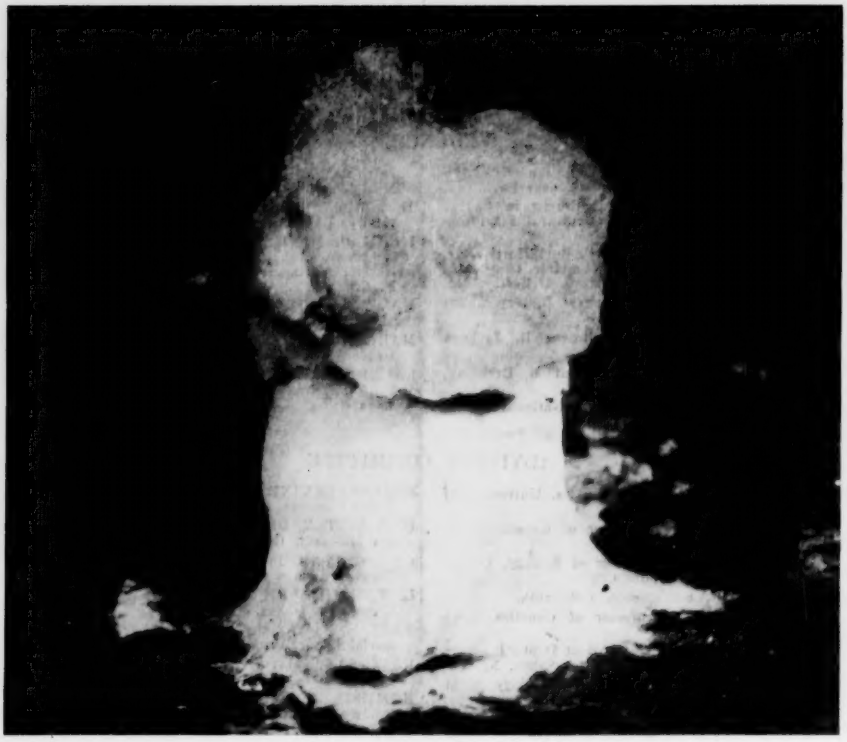

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Cytological Studies in Saccharum

A Quadruple Monosomic in Cotton

Some Striking Genetic Characters in Rice

New Evidence on Sex Determination in Honey Bees

VOLUME 48 JULY-AUGUST, 1957 NUMBER 4

The American Genetic Association

An incorporated organization devoted to promoting a knowledge of the laws of heredity and their application to the improvement of plants, animals, and human racial stocks

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QUADRUPLE MONOSOMIC PLANT OF *G. HIRSUTUM*

Figure 1

This plant, lacking four large chromosomes, was recovered in a progeny of a primitive introduction of *G. hirsutum*. The plant was vigorous, though sterile, and showed modification of plant habit, maturity and leaf structure.

A QUADRUPLE MONOSOMIC IN COTTON

P. A. Kammacher, M. S. Brown and J. S. Newman*

As one means of increasing the sources of new breeding material for cotton improvement at the Texas Agricultural Experiment Station, primitive stocks of *Gossypium hirsutum* have been introduced from their probable center of origin in southern Mexico. These introductions have been grown from seed under conditions prevailing at College Station, Texas to assess their possible economic value. Among the introductions collected by Drs. J. O. Ware and C. W. Manning in 1946 is a strain now known as Mexican 74A, which originated near Acala, Mexico. In a progeny of the original collection which was grown for evaluation in 1955, a single aberrant plant was found by J. S. Newman. This plant was marked by its compact bushy growth, and its thick, dark, slightly crinkled leaves. It was further distinguished by a completely monopodial branching habit. The other plants in the progeny row had the sympodial habit which is characteristic of the cultivated races of *G. hirsutum*. When the aberrant plant was first noticed it had not flowered, whereas sibling plants had already passed the peak of flowering and were setting bolls. Flowers first appeared on the aberrant plant early in August, when clusters of them were formed at the ends of the branches. Although the flowers appeared normal, no bolls were produced. In late August the plant was removed to the greenhouse for closer study of the nature of its sterility.

Under greenhouse conditions the plant retained its vegetative habit, but the new leaves were reduced to about one-third the original size (Figure 1). Numerous flowers, slightly smaller than normal and with incomplete anther dehiscence, again formed in compact clusters only at the ends of the branches. The fertility of the plant was tested in three types of crosses, as fellows: Self-pollination of the flowers produced no bolls. Use of the pollen on other lines of *G. hirsutum* was likewise unsuccessful. More than 100 flowers of the plant were hand-pollinated with pollen of fertile lines of *G. hirsutum*. From these, five abnormal three-loc bolls developed, which fell 10 days after pollination. These immature bolls contained swollen ovules which indicated that fertilization had taken place. Nevertheless, the plant proved to be completely male and female sterile.

Cytological Studies

Chromosome number and pairing—The altered morphology of the plant, coupled with its sterility, suggested chromosome aberration as the underlying cause. A study of meiotic behavior was therefore made in pollen-mother-cells. Young buds were fixed in acetic alcohol and the anther contents stained with aceto-carmine. At metaphase I of meiosis, 115 cells were analyzed as follows: 95 cells had 22 bivalents and four univalents (Figure 2), 13 cells had 21 bivalents and six univalents, seven cells had 20 bivalents and eight univalents. No multivalents were observed, except in one aberrant pollen-mother-cell which had 44 bivalents, two univalents and a chain of six chromosomes. The plant under investigation thus had only 48 chromosomes instead of the normal number of 52. Since the highest pairing recorded was 22 bivalents, the four missing chromosomes must have belonged to four different pairs; the plant therefore must be a quadruple monosomic, $2n$-1-1-1-1. The four monosomes were consistently large in size (Figure 2), which indicates that

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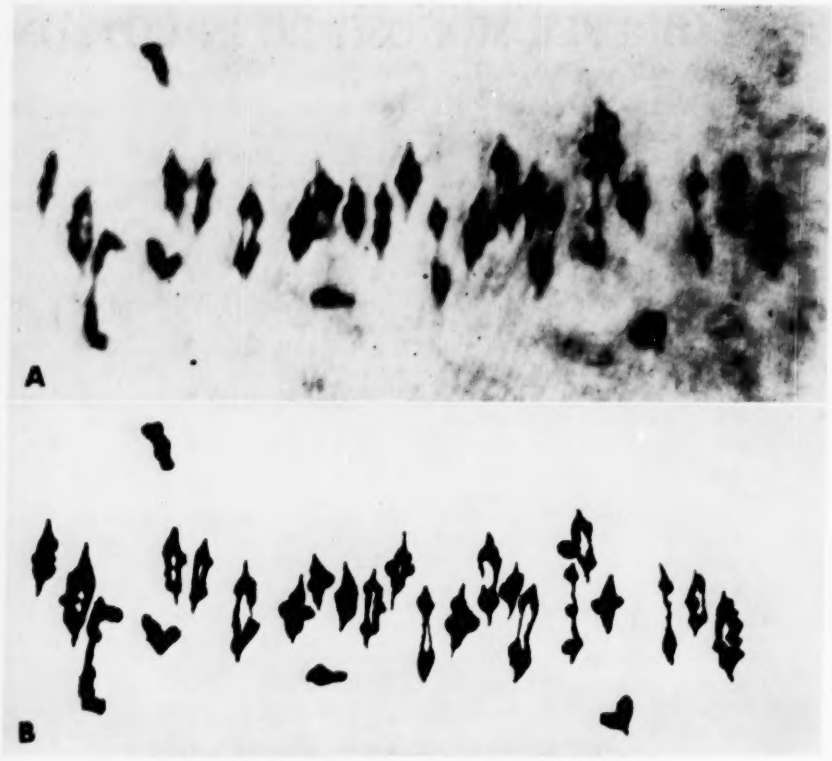

CHROMOSOMES OF A QUADRUPLE MONOSOMIC

Figure 2

A and *B* show the meiotic metaphase I of a quadruple monosomic with 22 bivalents and four univalents.

they probably belong to the *A* subgenome of *G. hirsutum.*

Chiasma frequency—The fact that about 17 percent of the pollen-mother-cells were found to have more than four univalents seemed to indicate that the loss of four chromosomes had reduced the degree of pairing in the diploid complement of the plant. To clarify this question the number of chiasmata was counted in 70 clear metaphase plates. It was found that out of a total of 1540 potential bivalents, 17, or 1.0 percent, were unpaired; 92, or 5.97 percent, had one chiasma; and 1431, or 92.93 percent, had two or more chiasmata, the latter appearing as "ring" bivalents. These results do not denote a marked decrease in the degree of pairing in comparison with the situation in normal *G. hirsutum,* in which a small proportion of rod bivalents always occurs. Only slightly more than one percent of the bivalents fail to pair at metaphase I, which indicates that the loss of four chromosomes has but little effect on the pairing of the rest of the chromosome complement of the plant. The slight reduction in pairing which is observed is not sufficient to be termed asynapsis.

Sporad analysis—To study the regularity of microspore formation, 416 sporads were examined. Of this number, 356 sporads, or 86 percent, contained

four microspores; the rest contained 2, 3, 5 or 6 microspores. However, the great majority of the four-spore sporads contained microspores of very unequal size. Some sporads contained four microspores more or less normal in size plus a very small microspore about one-fifth of the normal size. The great irregularity in microspore formation in contrast to the regularity of metaphase pairing is probably a consequence of the behavior of the univalents at anaphase I. If the distribution of the four univalents were at random at anaphase I, one pollen grain out of 16 would have 26 chromosomes and should be functional. However, the pollen tested was entirely sterile. Apparently genic deficiency, added to irregular chromosome distribution, contributed to the failure to form functional microspores.

Discussion

To our knowledge this is the first reported case of the spontaneous occurrence of a quadruple monosomic plant. The manner in which the plant arose is open to speculation. It is possible that the plant arose from the fusion of two normal gametes, and that the elimination of four chromosomes took place by misdivision in the nuclei of the cells in early stages of the development of the embryo. If this were the case one might expect the plant to be a mosaic of normal and monosomic tissue. However, the cytological analysis of buds collected from many branches gave no evidence of such mosaicism.

A more probable explanation for the origin of the multiple monosomic plant is suggested by the occurrence of the aberrant pollen-mother-cell mentioned above which had 44 bivalents, two univalents and a chain of six chromosomes. Such changes in chromosome number apparently occur naturally in *Gossypium*. Among all the field plants analyzed in a five-year period, Brown[1] and others found one or more aberrant pollen-mother-cells, with more or less than the *2n* chromosome number, in seven percent of the plants. Of 300 classified cells, 10 percent had less than the *2n* number of chromosomes. One deficient pollen-mother-cell has been reported in Asiatic cotton[3]. Male transmission of deficiencies has been demonstrated in *Gossypium* only for small segments of chromosomes[5]. However, large duplication-deficiencies from translocations are readily transmitted through the ovules[6], as are deficiencies of whole chromosomes (unpublished data of Brown and coworkers). If spontaneous changes in chromosome number such as occur in microsporogenesis occur also in macrosporogenesis, female gametes having less than the *n* number of chromosomes may be formed. Even if less frequent than aberrant male gametes, their greater tolerance for deficiencies would make them more likely to remain functional. Therefore, fertilization of a deficient egg cell by a normal male gamete, followed by normal development, could ensue. In such a manner the quadruple monosomic may have originated.

Absence of the four chromosomes produced both ovule and pollen sterility in the plant. It was therefore not possible to obtain from it a series of simple monosomics with which to test the effects of the chromosomes individually. Collectively, the absence of the chromosomes had a pronounced effect on plant morphology, which was intensified by the late maturity and sterility of the plant. The hemizygous state of the genes in the four chromosomes modified the type of branching, earliness, distribution of flowers and structure of leaves. These visible changes show that many developmental processes of the plant were altered by the deficiency of genic material. The genes involved are probably large in number, with little individual effect, possibly polygenic or modifier in nature, but essential for the reproductive process and unable to function in hemizygous condition.

Despite these pronounced changes in reproductive physiology and plant morphology, the vigor of the plant was not decreased. If genes affecting vigor are on these chromosomes, they can function at near normal capacity in hemizygous condition. This is consistent with the view that many genes affecting vigor of growth are dominant.

A second alternative is suggested by monosomic studies now in progress in this laboratory. Unpublished data of Brown and others show that monosomics involving large chromosomes are recovered and transmitted more frequently than those involving small chromosomes. Apparently *G. hirsutum* can tolerate the absence of certain large chromosomes with little effect on viability. The vigor of the quadruple monosomic is a striking confirmation of this fact.

Tests have verified that certain of the large simple monosomics are members of the *A* genome, as their size had indicated. Judged by their large size, the four chromosomes missing in the multiple monosomic plant also belong to the *A* genome. Either these chromosomes carry few genes essential for viability of the plant, or the missing genes are compensated for by duplication in the *D* genome, *G. hirsutum* having originated from *A* and *D* genome species.

Clausen[2] and Sears[7] have pointed out the extent to which duplication attributed to polyploidy influences the recovery of monosomic and nullosomic deficiencies in *Nicotiana* and *Triticum*. *G. hirsutum* being an allotetraploid like *N. tabacum*, it may be expected to tolerate deficiencies to a like degree, although both species have been subject to independent evolu-

tionary trends which tend to diploidize the species. Harland[4] showed that *G. hirsutum*, among other species of the genus, possesses a well balanced internal genic system with strong interdependence of its constituents. Further study of monosomics in this species should show to what extent initial duplication in the allotetraploid species has been offset by divergence in gene function.

Summary and Conclusions

A plant lacking four large chromosomes was recovered in the progeny of a primitive introduction of *Gossypium hirsutum*. The plant was vigorous, though sterile, and showed modification of plant habit, maturity, and leaf structure. Judged by the size of the monosomes, the missing chromosomes belong to the *A* genome. Either no genes for viability are on these chromosomes, or the missing genes are compensated for by duplication in the *D* genome, *G. hirsutum* being an allotetraploid originating from *A* and *D* species.

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A NOTE ON PROGENY TESTS

J. F. Kidwell and P. R. Ternan*

A PREVIOUS note[3] presented a simple general probability expression for determining the minimum number of progeny required to test a male for heterozygosity for a recessive autosomal gene. The expression was illustrated using the five percent level of significance and several of the more feasible test matings. Any significance level and any test mating may be used by substituting the appropriate values in the expression.

Hutt[2] pointed out that in many tests (e.g. mating to homozygous recessive females) relatively few additional progeny greatly reduce the probability of reaching an erroneous decision; i.e., deciding a male is homozygous dominant when, in fact, he is heterozygous. Hutt[2] also called attention to an extensive set of tables compiled by Warwick[4] which give, for nine Mendelian ratios, the probability of getting by chance any ratio observed in test groups of one to fifty individuals. The numbers needed to yield probabilities of $>.05$ or $>.01$ may be observed directly. Moreover, it is easily determined whether any given sample best fits an expected ratio of 3:1 or 2:1; of 1:1 or 9:7. Warwick's tables are of great value.

It has long been obvious that the probability that an individual is heterozygous may be determined from a knowledge of the genotype of its parents or the frequency of the gene in the population from which the individual was obtained. Except for matings producing probabilities of zero or one, these values are not especially useful in deciding the fate of a particular male. Berge[1] has stated that this probability influences the results of a progeny test, and has presented formulae for determining the probability that an individual is homozygous dominant that include it.

A progeny test of the type under consideration is designed to evaluate the probability of deciding an individual is homozygous dominant when, in fact, he is heterozygous. The decision is based entirely on the result of the test matings. Knowledge of probability of heterozygosity based on a knowledge of the parents' genotype is not applicable to the progeny test.

One aspect of the problem appears worthy of further brief comment. A situation may be encountered with multiparous animals in which it is necessary to test a male by mating him to a group of his daughters, full sibs or half sibs, and the exact genotype of any particular test female is unknown. She is either homozygous dominant or heterozygous. It becomes of interest to determine the minimum number of test females, n_1, and of testcross progeny per female, n_2, for proof at any desired probability level, or to determine the probability level for any partciular n_1 and n_2. The expression presented by Kidwell[3] is based on the assumption that n_2 is equal to one. It is obvious that the number of test females or progeny per test female is of no concern when the female test group consists of homozygous recessive or known heterozygous individuals. It is only the total number of phenotypically normal progeny in the absence of homozygous recessive progeny that is of importance in such test matings.

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The general problem may be stated as follows. An hypothesis, Ho, is formulated with respect to a given male, in this instance that he is heterozygous. It is desired to test Ho; i.e., determine whether to accept or reject it, and that the probability of an erroneous decision be no greater than some specified figure. The male will produce n_2 progeny from each of n_1 test females. Ho will be accepted if any homozygous recessive individuals appear among the n_2 progeny and rejected if none appear.

The following development assumes: (1) the dam(s) that produce test daughters are homozygous dominant. (2) Only one parent of the full sibs is heterozygous. (3) Only the common parent of the half sibs is heterozygous. Should any of the dams (assumption 1) or both parents (assumption 2) or parents other than the common one (assumption 3) be heterozygous, the precision of the test would be increased. Under these assumptions the n_1 and corresponding n_2 are the same for sire × daughter, full sib or half sib test matings. The probability that a particular test female is heterozygous is, under Ho, one half. The optimum value of n_1 and corresponding n_2 will be determined in each instance by such factors as litter size, gestation period, facilities, time and desired significance level.

Should any homozygous recessive individuals appear among the test progeny and Ho is accepted, there is, barring mutation, no error. If no homozygous recessive individuals appear among the n_2 test progeny and Ho is rejected, (i.e., it is decided the male is homozygous dominant) an erroneous decision is possible since he may, in fact, be heterozygous and none of the n_1 test females may be heterozygous; or if one or more are heterozygous they may not produce any homozygous recessive test progeny due to the vagaries of chance. It is desired to evaluate the probability of an erroneous decision for a given n_1 and n_2 or to determine the minimum n_1 and n_2 for a given probability of error.

An erroneous decision is reached if the male is heterozygous and none of the female test group are heterozygous, or if one is heterozygous but produces no homozygous recessive testcross progeny or two are heterozygous but produce no homozygous recessive testcross progeny, . . . or n_1 are heterozygous but produce no homozygous recessive testcross progeny. The probability of an erroneous decision is the sum of the probabilities of the occurances enumerated above. The general equation is:

$$P\{\text{Error}\} = \frac{1}{2^{n_1}}\left\{1 + C_1^{n_1}\left(\frac{3}{4}\right)^{n_2} + C_2^{n_1}\left(\frac{3}{4}\right)^{2n_2} + C_3^{n_1}\left(\frac{3}{4}\right)^{3n_2} + \cdots + C_{n_1-1}^{n_1}\left(\frac{3}{4}\right)^{(n_1-1)n_2} + \left(\frac{3}{4}\right)^{n_1 n_2}\right\} \cdots\cdots (1)$$

$$C_k^n = \frac{n!}{k!\,(n-k)!}$$

The C_k^n are the number of distinct combinations of n items taken k at a time and are, of course, the coefficients of the binomial expansion $(a + b)^n$.

Table I presents the probability of an erroneous decision for several values of n_1 and n_2. It should be noted that the probability of error will be greater than .05 for fewer than five test females and greater than .01 for fewer than seven test females, regardless of the number of testcross progeny per female. Two testcross progeny per female are required for 13 through 22 females, inclusive, to reduce the probability of error below .05. Equation one yields the same result as the expression presented by Kidwell[3] when n_2 is equal to one.

The chief utility of Table I is in designing a particular test. Assume, for example, one is working with a rat colony where the size of virtually all litters is at least six. If the five percent level were accepted, six test litters would be produced; if the one percent level were desired, nine litters would be produced. If some litters contained more or less than six, the precision of the test would be altered accordingly.

In actual practice the test females will rarely produce equal numbers of progeny, n_2. The computations for evaluating the probability of error in this case are quite tedious. In designing a test it is usually most practical to produce sufficient litters with at least the minimum n_2 for the desired probability level. Additional individuals will serve to lower the probability of error.

It may, however, be desired to evaluate the probability of an erroneous decision with unequal numbers of test progeny. The situation may be visualized as follows. The male is mated to n test females (daughters, half sibs or full sibs). Female 1 produces P_1 test progeny; female 2 produces P_2 test progeny . . . and female n produces P_n test progeny. An erroneous decision is reached if the male is heterozygous and none of the test females are heterozygous or if the male is heterozygous and one or more test females are heterozygous but no homozygous recessive offspring are produced. The probability of an erroneous decision is the probability that none of the test females are heterozygous plus the sum of the proabilities that each of the n females is heterozygous but none of the testcross progeny homozygous recessive plus, for all possible combinations of the n test females and their progeny taken 2, 3, . . . n at a time, the sum of the probabilities that all females in the

combined group are heterozygous but none of the test cross progeny are homozygous recessive. The general equation is:

$$P\{\text{Error}\} = \frac{1}{2^n}\left\{1 + \sum_{\alpha_1=1}^{n}\left(\frac{3}{4}\right)^{P\alpha_1} + \sum_{\alpha_2=1}^{n-1}\left(\frac{3}{4}\right)^{P\alpha_2}\sum_{\alpha_1=\alpha_2+1}^{n}\left(\frac{3}{4}\right)^{P\alpha_1} + \sum_{\alpha_3=1}^{n-2}\left(\frac{3}{4}\right)^{P\alpha_3}\sum_{\alpha_2=\alpha_3+1}^{n-1}\left(\frac{3}{4}\right)^{P\alpha_2}\sum_{\alpha_1=\alpha_2+1}^{n}\left(\frac{3}{4}\right)^{P\alpha_1} + \cdots\right.$$

$$\left. \cdots + \sum_{\alpha_n=1}^{1}\left(\frac{3}{4}\right)^{P\alpha_n}\sum_{\alpha_{n-1}=\alpha_n+1}^{2}\left(\frac{3}{4}\right)^{P\alpha_{n-1}}\sum_{\alpha_{n-2}=\alpha_{n-1}+1}^{3}\left(\frac{3}{4}\right)^{P\alpha_{n-2}}\cdots\cdots\sum_{\alpha_1=\alpha_2+1}^{n}\left(\frac{3}{4}\right)^{P\alpha_n}\right\} \quad \text{---------- 2}$$

Expressions involving two or more sigmas indicate multiple summations rather than the product of sums. A simple example might serve to clarify equation *2*. Consider, then, the following data:

Test Female, n.	1	2	3	4	5
Normal* testcross progeny, Pn	4	6	2	5	3

*No homozygous recessive testcross progeny appeared.

Equation two yields the same result as equation one when the test females produce equal numbers of testcross progeny.

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$$P\{\text{Error}\} = \frac{1}{2^8}\left\{1 + \left(\frac{3}{4}\right)^4 + \left(\frac{3}{4}\right)^6 + \left(\frac{3}{4}\right)^5 + \left(\frac{3}{4}\right)^3 + \left(\frac{3}{4}\right)^{4+6} + \left(\frac{3}{4}\right)^{4+2} + \left(\frac{3}{4}\right)^{4+5} + \left(\frac{3}{4}\right)^{4+3} + \left(\frac{3}{4}\right)^{6+2} + \left(\frac{3}{4}\right)^{6+5} + \left(\frac{3}{4}\right)^{6+3} + \left(\frac{3}{4}\right)^{2+5}\right.$$
$$+ \left(\frac{3}{4}\right)^{2+3} + \left(\frac{3}{4}\right)^{5+3} + \left(\frac{3}{4}\right)^{4+6+2} + \left(\frac{3}{4}\right)^{4+6+5} + \left(\frac{3}{4}\right)^{4+6+3} + \left(\frac{3}{4}\right)^{4+2+5} + \left(\frac{3}{4}\right)^{4+2+3} + \left(\frac{3}{4}\right)^{4+5+3} + \left(\frac{3}{4}\right)^{6+2+5}$$
$$+ \left(\frac{3}{4}\right)^{6+2+3} + \left(\frac{3}{4}\right)^{6+5+3} + \left(\frac{3}{4}\right)^{2+5+3} + \left(\frac{3}{4}\right)^{4+6+2+5} + \left(\frac{3}{4}\right)^{4+6+2+3} + \left(\frac{3}{4}\right)^{4+6+5+3} + \left(\frac{3}{4}\right)^{4+2+5+3} + \left(\frac{3}{4}\right)^{6+2+5+3}$$
$$\left. + \left(\frac{3}{4}\right)^{4+6+2+5+3}\right\}$$

$$P\{\text{Error}\} = 0.133$$

TABLE 1. The probability of no homozygous recessive individuals occurring among the n_2 progeny of a heterozygous male by each of n_1 normal daughters, (dams homozygous dominant) half sibs (only common parent heterozygous) or full sibs (only one parent heterozygous)

Normal testcross progeny, n_2, and no homozygous recessive progeny.	TEST FEMALES, n_1									
	5	6	7	8	9	10	11	12	13	14
1	.513	.449	.393	.344	.301	.263	.230	.201	.176	.154
2	.291	.227	.178	.139	.108	.085	.066	.052	.040	.032
3	.182	.129	.092	.065	.046	.033	.023	.017	.012	.008
4	.124	.081	.054	.035	.023	.015	.010	.007	.004	
5	.091	.056	.035	.021	.013	.008				
6	.071	.042	.025	.014	.009					
7	.058	.033	.019	.011						
8	.050	.028	.015	.008						
9	.045	.024	.013							

CYTOLOGICAL STUDIES IN SACCHARUM AND ALLIED GENERA

IV. Hybrids from *S. officinarum* ($2n = 80$) × *S. spontaneum* ($2n = 96$)

SAM PRICE*

CHROMOSOME numbers in *Saccharum officinarum* L. × *S. spontaneum* L. hybrids, with rare exceptions, have equaled the sum of the unreduced maternal chromosome number and the reduced paternal number ($2n + n$)[8]. Crossing 80-chromosome *S. officinarum*, clone Cana Blanca, with 96-chromosome *S. spontaneum*, clone Mandalay, produced several 128-chromosome ($2n + n$) hybrids and one 88-chromosome ($n + n$) hybrid. This progeny is of particular interest because the cross involved a 96-chromosome form of *S. spontaneum* for the first time, and because an opportunity is provided to study meiotic chromosome pairing in both $n + n$ and $2n + n$ hybrids.

Materials and Methods

The origin of the *S. officinarum* clone, Cana Blanca, is unknown. This "noble" sugarcane became widely distributed in the Americas during the early development of the sugar industry. It is said that this clone is called Lahaina in Hawaii, and Bourbon, Otaheite, and Vellai in other countries. Materials for cytological studies were taken from Cana Blanca (Importation No. 194) in the U. S. Department of Agriculture sugarcane collection at Canal Point, Florida. Cuttings were germinated, young shoots were fixed, and squashes of meristematic leaf tissue were prepared by a modification of a method already described[7].

Mandalay is one of two *S. spontaneum* clones collected near Mandalay, Burma, in 1929 by Mr. H. P. Agee and Dr. A. J. Mangelsdorf. (The other clone is named Burma.) A fixation taken in the Hawaiian Sugar Planters' Association sugarcane breeding collection provided pollen-mother-cells for cytological studies of Mandalay. Parts of the inflorescence were fixed in a mixture of ethyl alcohol and acetic acid (3:1) and were transferred later to 70 percent alcohol for storage. Iron-aceto-carmine squashes were prepared by the usual technique.

In most breeding collections Burmese clones of *S. spontaneum* bloom too early to be used in hybridization with *S. officinarum*. However, in 1952, when Mandalay was shedding pollen at Canal Point, Florida, Dr. E. W. Brandes learned that a noble cane was flowering in a farmer's field near Palmira, Colombia. Mandalay pollen was collected in Canal Point, desiccated, refrigerated, and carried by plane to Colombia by Dr. Brandes where it was dusted upon the flowers of Cana Blanca. There was no danger of contamination by foreign pollen, for no other canes were growing in the area. The assistance of Dr. Brandes is gratefully acknowledged for making the hybrids obtained available for cytological studies and for supplying some of the fixed materials. Fixations and squashes of the pollen-mother-cells of these hybrids were prepared as described above.

Observations

Several cells were seen in the leaf squash preparations of Cana Blanca, the *S. officinarum* parent, in which approximately 80 chromosomes could be counted. In two very clear cells it could be

*Geneticist, Crops Research Division, Agricultural Research Service, U.S.D.A., Beltsville, Maryland. Cytological investigations conducted as a cooperative project of the Hawaiian Sugar Planters' Association and the United States Department of Agriculture. Published with the approval of the Director as Paper No. 49 in the Journal Series of the Experiment Station, H.S.P.A., Honolulu, Hawaii. For reading the manuscript and for their suggestions, the author wishes to thank Drs. A. J.Mangelsdorf, G. L. Stebbins, and I. E. Stokes.

TABLE I. Chromosome association in hybrids from *Saccharum officinarum* (clone Cana Blanca) × *S. spontaneum* (clone Mandalay)

Hybrid	$2n$	Interpretation	Frequency of first metaphase associations							Total Cells
			44_{II}	$43_{II} + 2_I$	$42_{II} + 4_I$	$41_{II} + 6_I$	$40_{II} + 8_I$	$39_{II} + 10_I$		
P.B. 52-1-3	88	$n + n$	4	5	1					10
			64_{II}	$63_{II} + 2_I$	$62_{II} + 4_I$	$61_{II} + 6_I$	$60_{II} + 8_I$	$59_{II} + 10_I$		
P.B. 52-1-1	128	$2n$			2					2
P.B. 52-1-4	128	$2n$		5	4	1				10
P.B. 52-1-7	128	$2n$			4	2	1	1		8

CLONE CANA BLANCA

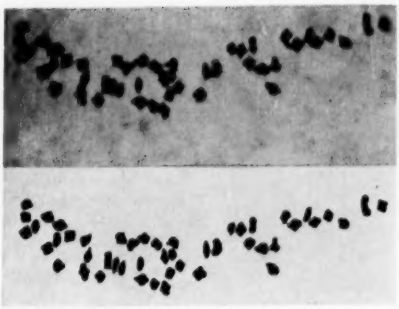
Figure 3

Late prophase in a leaf cell of *S. officinarum*, clone Cana Blanca. $2n = 80$. × 1300.

seen that $2n = 80$ in this noble cane (Figure 3).

In pollen-mother-cell divisions in Mandalay, the *S. spontaneum* parent, 48 bivalents were seen in seven cells at diakinesis. Two metaphase I cells also clearly showed 48 pairs (Figure 4). Although univalents were not clearly recognized, one first metaphase cell with 49 configurations probably contained 47 bivalents + 2 univalents.

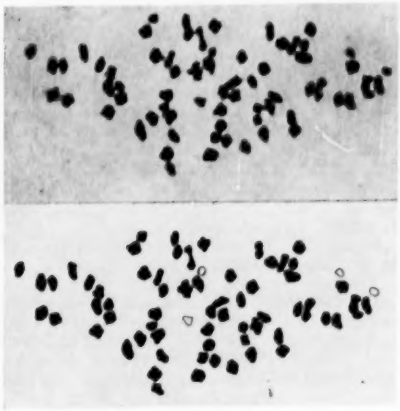
Observations on meiotic chromosome pairing in Cana Blanca × Mandalay hybrids are summarized in Table I. Occasional chromosome configurations were seen which resembled multivalents,



CLONE MANDALAY

Figure 4

Metaphase I in a pollen mother cell of *S. spontaneum*, clone Mandalay showing 48 bivalents. × 1000.



METAPHASE I—P.B. 52-1-1

Figure 5

Metaphase I in a pollen-mother-cell of the $2n + n$ hybrid, P.B. 52-1-1. 62 bivalents + 4 univalents. × 1000.

but these configurations appeared to be formed by "sticky" connections rather than by true chiasmata. Nishiyama[3] also noted "loose connections" responsible for multivalent-like chromosome associations in *Saccharum* hybrids.

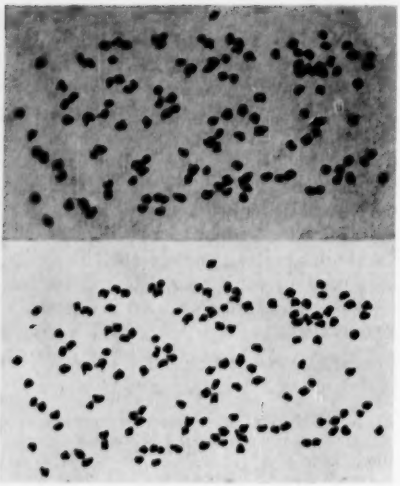
Meiosis was studied in three of the 128-chromosome ($2n + n$) hybrids (Figures 5 and 6). A total of 20 analyzable metaphase I cells were found. Cells with 2, 4, 6, 8, and 10 univalent chromosomes were seen. The most complete pairing observed, 63 bivalents + 2 univalents, was found in five cells. The most frequent configuration, 62 bivalents + 4 univalents, was seen in 10 cells.

In pollen-mother-cell preparations of P.B. 52-1-3, the 88-chromosome ($n + n$) hybrid, 10 metaphase I cells could be analyzed. (The initials P.B. designate varieties of the Palm Beach Research Farm, Canal Point, Florida.) The maximum pairing possible, 44 bivalents, was seen in four cells. The most frequent configuration was 43 bivalents + 2 univalents (Figure 7), and a single cell showed 42 bivalents + 4 univalents.

Discussion

The 40-chromosome gametic complement of *S. officinarum* is duplicated in the 128-chromosome ($2n + n$) hybrids. One might expect these duplicated chromosomes to pair among themselves, and such appears to be the case. If synapsis of *S. officinarum* chromosomes with those of *S. spontaneum* were of frequent occurrence, trivalents, each formed from two *S. officinarum* and one *S. spontaneum* chromosome, would probably be found in rather large numbers. The rarity or absence of multivalents (even "multivalent-like" configurations are rare) and the large number of bivalents in $2n + n$ hybrids (64 bivalents, the maximum possible association in pairs, are approached closely in three-fourths of the cells examined) suggest that allosyndesis is, at most, uncommon. It is supposed that formation of 64 bivalents by autosyndesis (40 bivalents among *S. officinarum* chromosomes and 24 bivalents among *S. spontaneum* chromosomes) is possible in these hybrids. The univalents that are seen may result from pairing failure among either *S. officinarum* or *S. spontaneum* chromosomes, but the latter, being present only in reduced number, are more likely to remain unpaired.

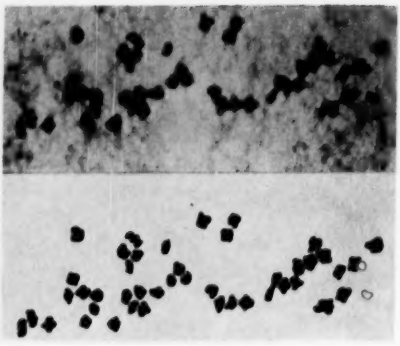
Meiotic chromosome configurations in $2n + n$ hybrids from *S. officinarum* crossed with cytologically different forms of *S. spontaneum* have generally received interpretations very much like the interpretation set forth above. That is, such hybrids are usually said to form 40 bivalents among the *S. officinarum* chromosomes and a variable number of bivalents and univalents among the *S. spontaneum* chromosomes. Implied in such interpretations is the supposition that pairing between *S. officinarum* and *S. spontaneum* chromosomes fails in $2n + n$ hybrids because *S. officinarum* chromosomes, being present in duplicate, pair preferentially among themselves. One might have supposed that chromosomes of the two species would pair with each other (allosyndesis) if only reduced complements were present. That such is not the



ANAPHASE I—P.B. 52-1-1

Figure 6

Anaphase I in a pollen-mother-cell of the $2n + n$ hybrid, P.B. 52-1-1. × 850.



***N + N* HYBRID**

Figure 7

Metaphase I in a pollen-mother-cell of the $n + n$ hybrid, P.B. 52-1-3. 43 bivalents + 2 univalents. × 950.

case, however, is demonstrated in pollen-mother-cells of the $n + n$ hybrid, P.B. 52-1-3.

Eight, 10, and 12 are the most prominently proposed basic chromosome numbers for *Saccharum*. The 40 chromosomes in the gametic complement of *S. officinarum* might consist of basic sets of 10 chromosomes (10 + 10 + 10 + 10) or of eight chromosomes (8 + 8 + 8 + 8 + 8). The reduced number of the 96-chromosome forms of *S. spontaneum* might be composed of sets of 12 chromosomes (12 + 12 + 12 + 12), sets of eight chromosomes (8 + 8 + 8 + 8 + 8 + 8), or, as Nishiyama[3] suggests, a combination of 8- and 10-chromosome sets (8 + 10 + 10 + 10 + 10). However, no combination of these schemes will account for the 44 bivalents observed here in the $n + n$ hybrid if an explanation is sought exclusively on the basis of allosyndesis. Combined autosyndesis and allosyndesis would probably result in numerous multivalents, and these are rare or absent in the hybrid under consideration. The 44 bivalents found in meiotic divisions in the 88-chromosome ($n + n$) hybrid are best explained as resulting from autosyndesis alone. Thus 20 bivalents are probably formed among the gametic chromosomes of *S. officinarum* and 24 bivalents are probably formed among the gametic chromosomes of *S. spontaneum*, clone Mandalay. The univalents seen in certain cells may be unpaired chromosomes from either species.

Alternate propositions concerning homologies are suggested: (1) So little homology exists between the chromosomes of *S. officinarum* and those of *S. spontaneum* that they are incapable of synaptic association, or (2) the chromosomes of *S. officinarum* and *S. spontaneum* are at least partially homologous but association between them is deferred in the $n + n$ hybrid because of preferential pairing among the gametic chromosomes of each species (autosyndesis). Under the first proposition, no genetic exchange between the chromosomes of the two species can be expected. Under the second proposition, genetic exchange, however rare, is possible. Unfortunately, the two alternatives are not resolved in the present study.

Whether or not homology between the chromosomes of the two species is finally demonstrated, formation of 44 bivalents by autosyndesis in the $n + n$ hybrid indicates that the gametic chromosomes of *S. officinarum* and of Mandalay *S. spontaneum* are, themselves, duplications. Since 20 of the 44 bivalents in the 88-chromosome hybrid result from pairing among the 40 gametic chromosomes of *S. officinarum*, those 40 chromosomes are shown to consist of two 20-chromosome groups. The capability of the gametic chromosomes of *S. officinarum* to form 20 bivalents by autosyndesis has also been shown in studies of *Saccharum officinarum* × *Narenga porphyrocoma* (Hance) Bor[1] and *Saccharum officinarum* × *Sclerostachya fusca* A. Camus[5]. How such observations support the idea that 10 is the basic chromosome number in *Saccharum officinarum* is discussed by Nishiyama[3].

Observations recorded here lend no support to previous suggestions regarding the nature of 96-chromosome forms of *S. spontaneum*. Janaki Ammal and Singh[2], studying root tips, found $2n = 96$ in a clone from Burma and suggested it may be a "triploid" of a form with $n = 32$. However, in studies of the clone Mandalay (and the clone Burma as well) the writer has found only bivalents and rarely

univalents, never trivalents. Nishiyama's studies[3,4] led him to propose that the 48 gametic chromosomes of Karenko, a 96-chromosome *S. spontaneum* clone from Taiwan, consist of one 8-chromosome set and four 10-chromosome sets (8 + 10 + 10 + 10 + 10). However, this proposal, if extended to the 96-chromosome clone, Mandalay, is in conflict with chromosome pairing observed in Cana Blanca × Mandalay hybrids. The 48 gametic chromosomes of Mandalay, if constituted according to Nishiyama's proposal, could not form 24 bivalents by autosyndesis. That they do pair among themselves to form 24 bivalents in the hybrid shows the reduced chromosome complement of Mandalay *S. spontaneum* to consist of two 24-chromosome groups. No evidence is available in the present study to suggest how the two 24-chromosome groups might be further subdivided into basic chromosome sets. Of the numbers proposed as basic for *Saccharum*, either eight or 12 would fit.

The fact that the chromosomes of the clone Mandalay can not fit the specific scheme proposed for a 96-chromosome *S. spontaneum* by Nishiyama need not be taken as evidence against the more general hypothesis that eight and 10 are the basic chromosome numbers in *Saccharum*[3,6]. On the basis of information now available in the literature, eight and 10, rather than 10 and 12, remain the more likely basic numbers for the genus. If eight and 10 are confirmed as the basic numbers in *Saccharum*, the *S. spontaneum* clone, Mandalay, will necessarily be interpreted as twelve-ploid with the basic number being eight.

Summary

Meiotic chromosome pairing is described for hybrids from 80-chromosome *Saccharum officinarum*, clone Cana Blanca × 96-chromosome *S. spontaneum*, clone Mandalay. The maximum association of chromosomes observed in 128-chromosome (*2n* + *n*) hybrids is 63 bivalents + 2 univalents. In an 88-chromosome (*n* + *n*) hybrid, 44 bivalents is the maximum chromosome association. There is no evidence of synaptic association between the chromosomes of *S. officinarum* and those of *S. spontaneum* in either *2n* + *n* or *n* + *n* hybrids. Autosyndesis in the hybrids indicates that the 40 gametic chromosomes of *S. officinarum* consist of two groups of 20, and, similarly, that the 48 gametic chromosomes of Mandalay, *S. spontaneum*, consist of two 24-chromosome groups. The proposal that 10 is the basic chromosome number in *S. officinarum* is supported. Either eight or 12 as basic chromosome numbers in the Mandalay clone of *S. spontaneum* would fit the data presented. Eight seems preferable, however.

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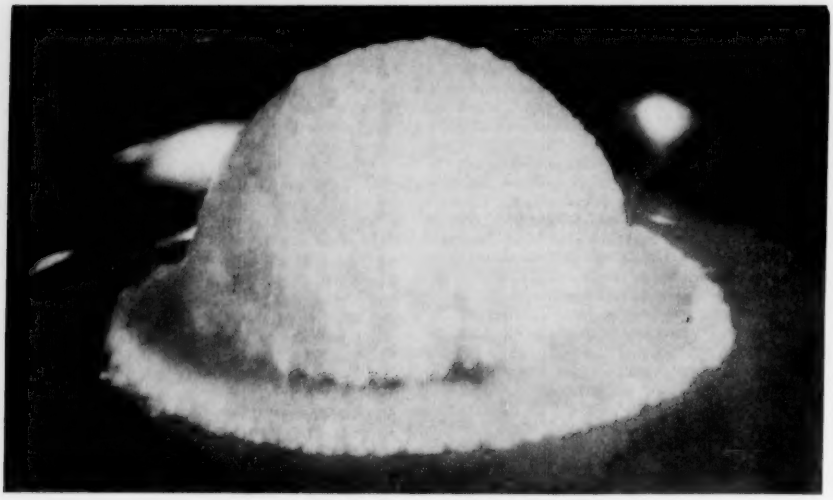
EXOTIC GROWTH FORMS IN NEUROSPORA

ADRIAN M. SRB*



MELON MUTANT OF *NEUROSPORA*

Figure 8

Melon cultures of the kind illustrated above tend to grow as hemispheres with a small platform of compacted hyphae beneath.

AFTER treatment of wild-type *Neurospora crassa* with beta-propiolactone, a variety of morphological mutants has been recovered[5]. Among the most striking are "melon" mutants, so-called because cultures derived by the germination of single ascospores are initially almost spherical in form, have a tough outer rind of compacted hyphae, and are light orange in color. Inheritance studies show melons and wild type to differ by a single allelic alternative.

Since the *Neurospora* melons are aconidial, vegetative reproduction of cultures is accomplished by the transfer of small chunks of the compacted hyphal mass. Cultures reproduced in this way show the same restricted growth habit and other general features as the originals but tend to grow as hemispheres, sometimes with a small platform of compacted hyphae beneath (Figure 8). Such variations from the prototype appear to be nongenetic, since after a cross typical melons are recovered from germinated ascospores.

Occasionally, melon cultures show variations in form that go beyond the restrictions usually imposed by their standard genotype. In such instances, exceedingly exotic growth forms are observed. These variant melon cultures show unusual vertical growth. The terminal hyphae ultimately occur in relatively loose association and extend laterally beyond the base of the culture. The general external form is grossly that of a mushroom (Figure 9).

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"MUSHROOM" FORM OF A MELON CULTURE

Figure 9

Melon cultures like that shown in Figure 8 sometimes undergo spontaneous transformation to forms like the one shown here. See text for details.

The present paper is concerned with genetic phenomena found to be associated with these remarkable variations in form.

Materials and Methods

The melon mutants and biochemical mutants of *Neurospora crassa* utilized in this study derive from beta-propiolactone treated conidia of wild-type strain 74A. This strain, isolated by P. St. Lawrence, was provided to us by D. Bonner, of Yale University. Strain 74A and its homologue of opposite mating type, 77a, were the wild-type strains utilized in the crossing experiments. The B. F. Goodrich Chemical Co. kindly supplied the beta-propiolactone. The method of isolation of mutants was essentially that described by Woodward, *et al.*[7]. Although this method was designed particularly for the recovery of mutants with nutritional deficiencies, morphological mutants able to grow on minimal medium are also recovered in relatively high frequency. Presumably this is because certain morphological mutants of restricted growth habit, melons among them, tend initially to grow as compact pellets in liquid media; thus they are able to pass through the cheesecloth filter whose function is to separate germinated wild-type conidia from nongerminated biochemical mutant conidia.

The media for culturing *Neurospora* were, unless otherwise stated, those de-

scribed by Beadle and Tatum[2]. Cultures to be observed for external form were grown on media solidified with 2.5 percent agar. The 10 × 75 mm. tubes ordinarily employed for keeping *Neurospora* cultures are too small to permit the kind of elongation typical of the mushroom forms of melon cultures. Observations of these variants were made on cultures grown in 125 ml. Erlenmeyer flasks or in 18 × 150 mm. culture tubes.

When grown by themselves neither melons nor the mushroom forms of melons produce conidia or protoperithecia. Crosses involving such a strain were started by placing chunks of its mycelium onto Difco Cornmeal Agar or Westergaard's medium[6], and then inoculating the other parent onto the same agar slant.

Observations

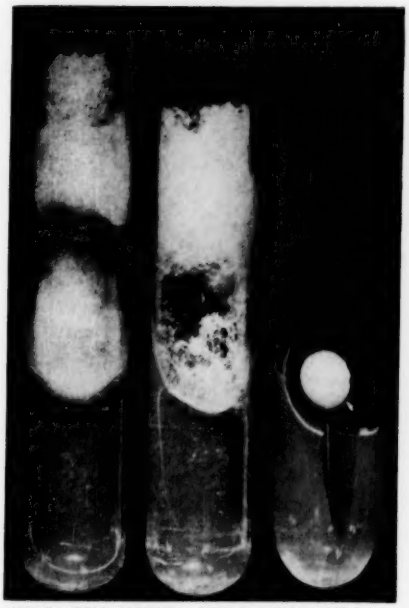
The results of crosses between *Neurospora* melons and strains having wild-type morphology show that the melon character is inherited on the basis of determination by a single nuclear gene. When such crosses are made, only melon and wild type morphologies are represented among the progeny. These occur in reasonable approximation of an expected 1:1 ratio, when randomly collected single ascospores are germinated. Table I summarizes results of two such crosses. As indicated for Cross I, results were obtained both by plating of ascospores and by isolating individual ascospores into separate culture tubes. The results are uniform in showing a deficiency of melon progeny, but significant deviation from a 1:1 ratio is found only where the data were obtained from platings of ascospores. The difference is possibly explicable on the basis that melons grow up slowly, and tend to be overrun by wild-type segregants occurring on the same plates. Where ascospores were isolated into separate tubes, the germination ranged between 70 and 80 percent.

When spores are dissected in order out of asci formed after crosses between melons and wild type, the results are entirely consistent with expectation based on single-gene segregation within the asci. The spores of 40 asci were isolated according to their linear order within the ascus. Of the 30 asci from which at least one member of each spore pair germinated, 18 showed the melon allele to segregate at the second division of meiosis. The result indicates the map position of this allele to be about 30 units from the centromere of its chromosome. The linkage relations of the melon locus have not been determined, but segregation is independent of adenine purple, known to be in linkage group I[1]. From a cross of purple with melon, 150 randomly collected ascospores were isolated. The resultant cultures included 34 wild, 27 purple, 29 melon, and 25 purple melon. Similar results were obtained from crosses between melon and an albino mutant situated in the same linkage group as adenine purple.

Melon cultures grow on the minimal medium for *Neurospora*. Neither their growth rate nor morphology has been found to be appreciably affected by nutritional supplements of hydrolyzed casein, or of the vitamins or purines standardly included in test media for *Neurospora*. On various complete media, containing supplements of yeast extract, malt extract, tomato juice, coconut milk, or hydrolyzed mycelium of wild-type *Neurospora*, melon cultures grow somewhat more rapidly than on minimal medium but their typical external form is maintained.

TABLE I. Results of crossing melon with wild-type *Neurospora*

Cross	Melon Progeny	Wild-type Progeny	χ^2	P
I (spores plated)	101	135	4.90	.03
I (spores isolated)	96	113	1.38	.30
II (spores isolated)	120	130	.40	.50
Total	317	378	5.35	.02



PROGENY TYPES FROM THE CROSS WILD TYPE × "MUSHROOM"

Figure 10

Wild type is in the center, with aconidial and melon on either side. The expected type, aconidial-melon either does not emerge from the cross or has gone undetected.

Occasionally, and apparently independently of the kind of media on which they are grown, melon cultures undergo bizarre phenotypic transformations of the kind already indicated. The ultimate form of the culture is, roughly speaking, mushroom shaped, a form generally preserved until the medium becomes so dry that the culture is discarded. In no instances have the kinds of external change involved in the mushroom effect been observed in vegetative cultures less than three weeks old. The frequency of occurrence of these altered melons is not accurately known. Seven instances have been observed in the 410 cultures grown under conditions where the morphological changes under discussion would be observable.

The first observed mushroom form of a melon culture was crossed with wild-type *Neurospora*. Two crosses were made. In Cross A, the inoculum of the variant parent was a chunk taken from near the base of the mushroom culture. The perithecia resulting from this cross yielded only a small number of well developed ascospores. Germination of these gave 71 wild-type and 58 melons, a result consistent with those obtained when wild-type is crossed wtih standard melon. In Cross B, the inoculum of the variant parent was a chunk of mycelium taken from the terminal portion of the mushroom. Again the cross yielded few ascospores, which gave but 23 percent germination. The progeny cultures included 56 wild types, 47 melons, and 32 isolates that resembled wild type in general form, including spreading growth habit, but were acodidial. Figure 10 shows the three types of culture found in this progeny.

The aconidial cultures maintain their phenotype through an indefinite series of asexual transfers. When crossed with each other they yield only aconidial progeny. When crosses are made with wild type, the progeny ascospores, isolated at random, give only wild-type and aconidial cultures, in an approximation of the 1:1 ratio expected if the morphologies of the parent strains depended upon a single allelic difference. For example, from a population of 450 ascospores isolated at random, 224 wild-type and 193 aconidial cultures were recovered. Assuming a 1:1 ratio, the chi-square for these data is 2.16, and the p-value 0.15. Analysis of 30 asci from which the spores were dissected in order confirmed that the aconidial character is the effect of a single gene, and indicated this gene to be about 16 map units from the centromere of its chromosome. Aconidial, like melon, segregates independently of adenine purple.

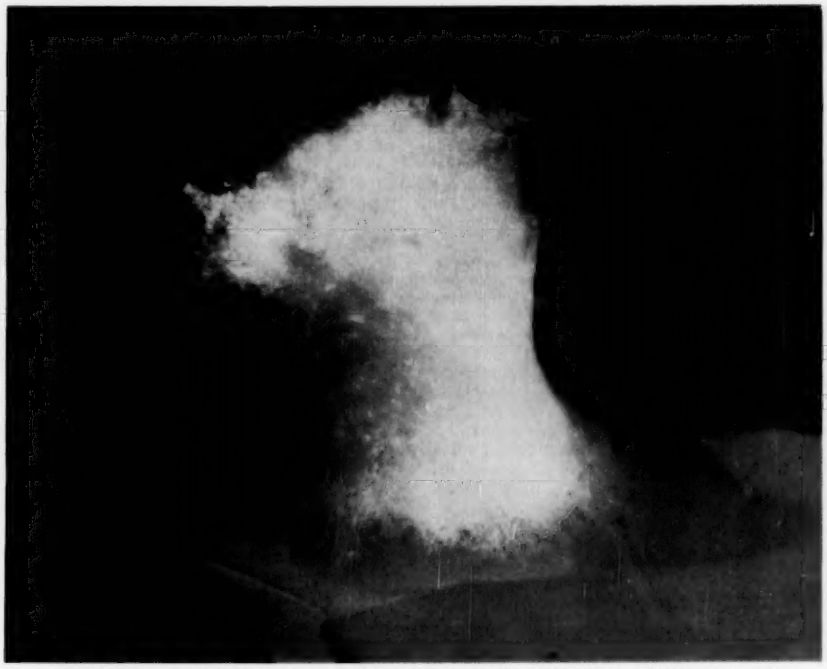
Apart from data permitting estimates of distance between gene locus and centromere, evidence that aconidial and melon are not allelic can be drawn from the results of crosses between these variant forms. The perithecia formed when a melon is crossed with aconidial are not very fertile, and of the spores formed

many are light colored and fail to germinate. Because of this high degree of sterility no tetrad analyses have been possible, but among ascospores isolated at random a high proportion of those that germinated gave wild-type cultures. An unexpected result of these crosses is that the melon parent was not recovered among the progeny. Neither was the possible double mutant, aconidial-melon, recognized. However, aconidial was recovered with roughly the frequency of the presumed wild-type recombinant. From crosses of melon with aconidial, 68 wild-type progeny and 63 aconidial progeny were obtained, with 32 percent germination of the spores isolated. Ten each of these wild-type and aconidial cultures were randomly chosen and then used as parents in crosses with wild-type strain 74A or 77a. The progeny wild-type and aconidial cultures, when used as parents, behaved as standard wild-types or aconidials, giving results of the kind already indicated.

The other mushroom-like cultures that have arisen from *Neurospora* melons resemble genetically the one already described. That is, when such cultures are crossed with wild type, aconidial as well as melon and wild-type progeny are recovered. Since the evidence indicates the origin of mushroom cultures to depend upon mutation giving rise to the gene for aconidial, the terminal portions of these cultures might be expected perhaps to be genetically inhomogeneous. In an effort to illuminate the genetics of these cultures as exposed by crosses with wild type, progenies deriving from particular perithecia were separately analyzed. Table II shows the results by perithecium from a cross of this kind. The results confirm that mushroom cultures contain aconidial as well as melon genes, but the number of spores was so small and the germination percentage so low that further conclusions must be tentative.

Some attempts were made to determine whether the terminal portions of mushroom cultures are heterocaryotic. The experiments are difficult because the cultures do not produce conidia and because the compacted mycelia offer few free hyphal tips. However, when chunks of a mushroom culture were flattened on an agar plate of minimal medium the early stages of ensuing growth gave free hyphal tips that could be cut off, isolated, and grown up in culture. From such a culture, hyphal tips were isolated again. The vast majority of isolates grew into cultures that were neither precisely like melon nor standard aconidial. Growth habit was not restricted as with melons, the hyphae were more compacted than is typical for aconidial, no conidia were formed, and the mycelium had a smooth, shiny appearance. On crossing with wild type, these cultures behaved like chunks from the terminal portions of mushroom cultures, giving melon, aconidial, and wild-type progeny. Two hyphal tip isolates gave cultures that were like melon in phenotype, and behaved like melon in crosses with wild type. The conclusion would appear to be that some heterocaryosis occurs in the terminal portions of mushroom cultures but that it may be rare.

Since the double mutant aconidial-melon could not be obtained, or at least was not recognized, studies of the interaction between these genes were made by means of experimentally produced heterocaryons. Heterocaryons between aconidial and melon were forced[3] by marking these cultures with different biochemical mutant genes and inoculating the different double mutant strains together on solid minimal medium. Usually heterocaryons failed to form, but several successes were obtained. Figure 11 shows a heterocaryon between an arginine-requiring melon and a methionine-requiring aconidial strain, grown on a medium deficient in these growth factors. This culture was proved to contain heterocaryotic hyphae by standard techniques involving the isolation of single hyphal tips. A heterocaryon involving a purple, adenine-requiring melon and an arginine-requiring aconidial culture gave a similar picture. It is of interest that forced heterocaryons be-



ATTEMPT TO PRODUCE A *NEUROSPORA* "MUSHROOM" ARTIFICIALLY

Figure 11

Above is a forced heterocaryon whose component nuclei derive from an arginine-requiring melon strain and from a methionine-requiring aconidial strain. The double mutants were isolated after appropriate crosses. The heterocaryotic culture was grown on a medium lacking arginine and methionine.

tween melons and strains with wild-type morphology have never given columnar type, vertical growth, but always have produced mycelia whose growth was largely lateral over the surface of their medium.

Discussion

A significant aspect of the present investigation is its emphasis that profound alterations in form are determinable by one or two gene mutations. The extremeness of possible deviation from the species phenotype can be appreciated when wild-type *Neurospora* is compared with melon, and with the mushroom form of a melon culture (Figures 8, 9 and 10). The melon phenotype represents a standard instance of single gene inheritance. When the effects of an additional mutation, presumably occurring spontaneously, are superimposed upon a growing melon culture, an interaction occurs that is observable as the mushroom form.

Whether the interaction inheres primarily in a double mutant nucleus, aconidial-melon, or depends upon heterocaryotic relationships between melon nuclei of the original culture type and nuclei containing a newly arisen mutation for the nonallelic gene for aconidial, is not clear. The terminal portions of mushroom cultures do include two kinds of nuclei, those that are single mutant melon and those carrying the gene for aconidial. The former have been recognized in the experiments involving isolation of hyphal tips. The latter are indicated by the crossing experiments with wild type, where wild, aconidial, and melon progeny appear. That these latter nuclei are indeed double mutant aconidial-melon is likely on two bases. First, the aconidial and melon genes are situated at different loci, and for the nuclei in question to be simply aconidial, rather than aconidial-melon, two gene mutations would have had to occur. Second, a not unlikely assumption permits interpretation of the genetic data on the basis that double mutant, aconidial-melon nuclei are present. The data derived from analysis of particular perithecia (Table II) show the three progeny

types, wild, aconidial, and melon, in approximately equal numbers. Such a result would be expected if the loci for melon and for aconidial were genetically independent and if the double mutant aconidial-melon were unable to survive some phase of the sexual cycle. Under this kind of interpretation, spores from perithecia A, B, D, E, F, G, H would seem to represent progeny derived after fusion of wild-type nuclei with aconidial-melon nuclei. Spores from perithecium I, and possibly C, might represent progeny derived after nuclear fusion between wild-type and melon nuclei. The interpretation must remain tentative because infertility of the perithecia resulted in a paucity of data, and because of Sansome's demonstration that nuclei of ascogeneous hyphae in a perithecium of *N. crassa* may come from several initial pairs of nuclei[4].

The major assumption of the interpretation is that certain nuclei carrying the melon gene do not pass through the sexual cycle. Such a tendency is indicated in simple crosses involving standard melon and wild type (Table I). In interpreting the results of crosses involving components of the mushroom forms it needs to be supposed that when wild type is crossed with aconidial-melon, the melon gene does not pass through the sexual cycle in combination with aconidial. Further, when the same genes are involved, but neither parent is a wild-type combination, i.e., when the cross is aconidial by melon, melon genes do not pass through the sexual cycle at all.

That interaction affecting form does occur between melon and aconidial genes is clear not only from genetic analysis of spontaneously occurring mushroom cultures but also from observation of the growth forms resulting when heterocaryons are forced between melon and aconidial strains. The experimentally produced heterocaryons do not in fact precisely resemble the spontaneously occurring mushrooms, as can be seen from comparison of Figures 9 and 11. The primary difference appears to be that in the former the hyphae are considerably more loosely associated. But precise resemblance is hardly to be expected, since the spontaneous mushrooms are given initial form by the properties of a pure melon culture, and since the heterocaryotic combination of spontaneous mushrooms appears to involve melon and melon-aconidial nuclei while that of the experimental mushrooms involves melon and aconidial nuclei.

The results should not leave the impression that a mushroom form appears whenever a mutation to the aconidial gene occurs within a melon culture. This proposition can not be tested. Spontaneous appearance of aconidials in cultures of *Neurospora* of wild-type growth habits is a rather commonly observed phenomenon, which would appear to mean either that the mutation is frequent, or that the mutant gene has selective advantages. In either instance, the occurrence of mutations to aconidial in melon cultures is not surprising. Whether the mutation occurs in melon cultures that retain their standard form is unknown.

Summary

Melon mutants of *Neurospora crassa* are striking variants from the wild type growth habit. Melon and wild type differ by a single nuclear gene. Melon cultures sometimes develop into mushroom-shaped forms. Correlated with these alterations in form is the appearance of an additional mutant gene, nonallelic to the gene that determines melon. This second mutant gene on a background of otherwise wild-type *Neurospora* determines an aconidial phenotype. The mushroom forms of melon cultures contain at least some heterocaryotic hyphae that include nuclei of the original melon type and nuclei that also carry the gene for aconidial. Experimentally produced heterocaryons involving a combination of nuclei carrying the melon gene and nuclei carrying the aconidial gene sometimes grow into forms resembling the spontaneous mushroom cultures, but not completely. After crosses where the fusion nuclei are expected to be heterozygous at both the loci for melon and aconidial, certain genotypic combinations involving melon are not recovered among the viable progeny.

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TABLE II. Results by perithecium of cross between wild-type *Neurospora* and "mushroom"

	Progeny			
Perithecium	Wild	Melon	Aconidial	Nongerminated
A	9	7	8	20
B	7	7	3	16
C	0	3	0	5
D	4	8	5	15
E	3	3	3	15
F	2	3	5	13
G	3	3	3	8
H	3	1	4	16
I	8	11	0	10
Total	39	46	31	118

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CYTOLOGY AND CYTOGENETICS

TWENTY years have passed since the publication of the last comprehensive text or reference book in the English language on cytology and cytogenetics; namely, the second edition of Darlington's "Recent Advances in Cytology." The need for such a book has been keenly felt for a long time by both research workers and teachers in the field. This need has now been met in splendid fashion.* Professor Swanson, whose extensive, original, and forward looking research on the effects of radiations on chromosomes has for some time attracted the interest of specialists in this field, has given us a book which is clearly written, magnificently illustrated, broad in scope, and modern in conception.

After two introductory chapters, one on modern techniques and one on the generalized cell, the author devotes the remaining sixteen chapters to a study of chromosomes as bearers of heredity, and to the impact of new discoveries about these structures on our concepts of genetics and evolution. When discussing those facts which are recognized by all cytologists, such as the general features of meiosis, the nature of crossing-over, and the principal variations in chromosome number and structure, Professor Swanson gives a clear, precise, and well illustrated description, designed to impress on the student the most important points. If, on the other hand, he is discussing a controversial matter, such as the number of chromonemata in a chromosome or the mechanism of crossing-over, he lists the principal theories which have been advanced, the arguments for and against each one of them, and the reasons why he favors a particular hypothesis or is unable to take a definite stand. Although such an approach might appear commonplace to scientists familiar with textbooks in other fields, it is nevertheless unusual in chromosome cytology. Most of the previous attempts to synthesize information in this field have been marred either by special pleading for a particular set of hypotheses, which has failed even to recognize the evidence against them and has inextricably confused fact and hypothesis, or by a too general approach, which has glossed over and failed to analyze the important differences in theory held by different cytologists.

The modern character of Professor Swanson's approach to his field exists throughout the book, but is particularly evident in his chapters on "Spontaneous and Induced Chromosomal Aberrations," "The Chemistry of Nuclei and Chromosomes," and "Chromosomes and Genes." After studying the careful analysis and partial synthesis of the extensive recent literature which he presents in these chapters, this reviewer gained the impression that biologists and biochemists are converging from three different directions on one of

**Cytology and Cytogenetics*. Carl P. Swanson. i-x, 1-596, 222 figs. Prentice-Hall, Inc., New York. 1957. $10.00.

the central problems of biology; the nature, organization, and mode of reproduction of the hereditary materials, or genes. The cytogenetic approach, exemplified by the pioneer work of McClintock on the breakage-bridge fusion cycle in maize and the more recent discoveries of Lewis, Green and others on pseudoallelism in *Drosophila*, is correlating chromosomal changes with their genetic effects on a relatively gross, microscopic level. The structure of chromosomes at the same level is being attacked from another direction by Professor Swanson himself, as well as by Hollaender and his co-workers, Kaufmann, and many others. By studying not only the direct effects of various types of radiations and chemical substances on chromosome breakage, but also the influence on this process of various types of physicochemical environment of the nucleus and chromosomes, they are discovering various ways in which the structure and metabolism of cells and chromosomes may be upset, and this is leading toward an understanding of these metabolic processes themselves. Finally, the submicroscopic structure and chemical composition of chromosomes and genes is being attacked by a variety of biochemical techniques; staining reactions, photometric procedures, enzyme digestion, and direct chemical analysis; all of which are described, with examples, by Professor Swanson. From these analyses, a tentative conception is being obtained of gene structure at the molecular level, exemplified by the Watson-Crick model. These three directions of approach have not progressed far enough toward each other so that they can be integrated into a complete hypothesis of the physicochemical structure of genes and chromosomes. Professor Swanson is to be commended on the way in which he has presented the major problems involved and the progress which has been made toward their solution, without attempting to make speculative hypotheses which go beyond the data.

The five chapters which complete the book, except for a short concluding statement, deal with certain aspects of cytology in relation to evolution. Here again, Professor Swanson reviews the most significant facts which are known and points out the major unsolved problems, without attempting to advance integrating hypotheses. One of the few criticisms which could be offered of the book concerns the title of one of the chapters, "Role of Aberrations in Evolution." Chromosomal differences such as inversions and translocations are so widespread and abundant in natural populations of both animals and plants, as Professor Swanson clearly shows, and play such a significant role in determining the way in which their genetic variability is made available for the action of natural selection, that they can hardly be regarded altogether as aberrations.

Cytologists, cytogeneticists, and biologists in general will be grateful to Professor Swanson for producing such a clear, comprehensive, and stimulating survey of his field. It will be a valuable reference work for many years to come.

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CHROMOSOME BREAKAGE INDUCED BY X-RAYS, MALEIC HYDRAZIDE AND ITS DERIVATIVES

In Relation to Knob Number in Maize

G. E. GRAF*

IN 1948 Ford[4] proposed that NN-di (2-chloro-ethyl) methylamine did not break *Vicia* chromosomes at random but rather showed some specificity for heterochromatin. This hypothesis was based on the distribution of breaks within the chromosome complement. The non-randomness of this breakage was confirmed by Loveless and Revell[9]. Using two purine derivatives, ethoxycaffeine and tetramethyl uric acid, Kihlman and Levan[6] found apparent localization of chromosome breaks in the attachment thread of the satellite on the long chromosome of *Vicia*. Revell[13] has compared the distribution of chromosome breaks in *Vicia* induced by various chemicals with that induced by X-rays and finds that they are different. In the chemically treated material the proportion of breaks at or near demonstrable heterochromatin was much greater than that expected by chance, although chemically induced breaks were clearly not confined to heterochromatic regions of the chromosomes. Oelkers[11] and coworkers have demonstrated chemically induced non-random breakage in *Vicia* root tips and in *Oenothera* meiotic cells.

Darlington and McLeish[3] reported that maleic hydrazide (2:6 pyridazine diol, henceforth to be referred to as MH) causes chromosome breakage in some plants and furthermore that this breakage appeared to be associated with heterochromatic chromosome regions. Broken chromosomes do not appear except with very low frequency in MH treated material until about 12 hours after the initiation of treatment[10]. This would indicate that the action of MH is on interphase nuclei and coincides quite closely with the time of desoxyribose nuclei acid synthesis as determined in *Vicia* by Howard and Pelc[5].

Carlson[2] has demonstrated that MH causes chromosome breakage in mitotic tissues of oats, soybeans, and corn. He also made the interesting observation that MH causes an increase in nucleolar volume and causes nucleoli to persist through mitosis.

In order to test the relationship between chemically induced chromosome breakage and heterochromatin, most workers have correlated the locations of breaks within the chromosome complement with the locations of heterochromatin. It appeared to be of some interest to test the hypothesis of preferential breakage in heterochromatin by utilizing variable amounts of heterochromatin in the chromosomes of a single species and studying the relationship between heterochromatin content and chromosome breakage. In corn this is, to some extent, possible owing to the occurrence of darkly-staining enlargements generally known as heterochromatic knobs. The number of knobs varies among varieties but is constant for individual varieties. Brown[1] has determined the knob number of numerous varieties of corn and finds that the number may vary from zero to 14 in varieties of United States corn. By using varieties of corn with different

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numbers of knobs one can thus, to some extent, manipulate the heterochromatin content.

Materials and Methods

Four varieties of corn (*Zea mays*) were tested for response to MH, MH derivatives, and X-rays. The corn consisted of two open-pollinated varieties, a knobless flint and Zapalote Chico (kindly provided by Dr. William Brown of the Pioneer Hybrid Corn Company) and two corn belt inbreds, HY and IDT. The knobless flint and Zapalote Chico have zero and 12 knobs respectively, while the IDT and HY have four and six knobs respectively[1]. The kernels were germinated routinely on moistened filter paper in the dark. After the primary roots were approximately two centimeters long, 10 to 20 seedlings were transferred to a petri plate containing ten cubic centimeters of chemical solution, or in the case of controls, distilled water, for the duration of treatment. The seedlings were then rinsed thoroughly and transferred back to petri plates containing filter paper moistened with tap water. The seedlings were kept in the dark except during rinsing and transfers. After the desired recovery period the roots were excised and fixed in a mixture of three parts 95 percent ethanol, one part glacial acetic acid. Aceto-carmine squash preparations were used routinely for cytological examination of the root tips.

Since the kernels of the different varieties could be distinguished morphologically with little difficulty, they were carried through the treatment and recovery periods in the same petri plates routinely so as to keep the conditions for the different varieties as uniform as possible. As peak chromosome breakage occurs approximately 24 hours after the initiation of treatment with MH, experiments were carried out with two hours of treatment followed by 22 hours recovery time in tap water. In addition to MH, N-acetyl MH, N-caproyl MH, and N- benzoyl MH were tested. (The MH and MH derivatives were kindly provided by Dr. J. W. Zukel of the Naugatuck Chemical Company). The solutions of MH derivatives were freshly prepared for each experiment.

Irradiation was carried out on roots one to three centimeters long. The seedlings were irradiated wet in air. The dosages used were 100 roentgens and 250 roentgens administered at a rate of 200 roentgens per minute, at a distance of 41 centimeters. A 0.25 millimeter copper and a 1.0 millimeter aluminum filter were used. The roots were fixed 22 hours after irradiation.

Rather than attempt to score chromosome breaks directly, breakage was indirectly measured in terms of anaphase bridges. Scoring was carried out in terms of percent anaphases with bridges. Cells with numerous bridges, which were common, thus were scored the

TABLE I. Comparison of chromosome breakage induced by chemicals and X-rays in four varieties of maize

		Percent anaphases with bridges Number of anaphases examined in parenthesis			
Treatment	Strain	KF	IDT	HY	ZC
	Knobs	0	4	6	12
Control		0 (100)	0 (100)	0 (100)	0 (100)
10^{-3} M MH		8.3 (156)	12.1 (99)	20.5 (83)	43.0 (210)
10^{-4} M MH		2.9 (242)	5.3 (207)	8.3 (120)	14.2 (338)
10^{-3} M N-acetyl MH		3.7 (54)	8.5 (152)	14.0 (93)	25.1 (175)
10^{-5} M N-benzoyl MH		3.2 (220)			23.5 (179)
100 Roentgens		2.6 (196)	3.7 (108)	2.5 (201)	1.1 (191)
250 Roentgens		10.2 (166)	7.9 (139)	9.1 (187)	8.8 (216)

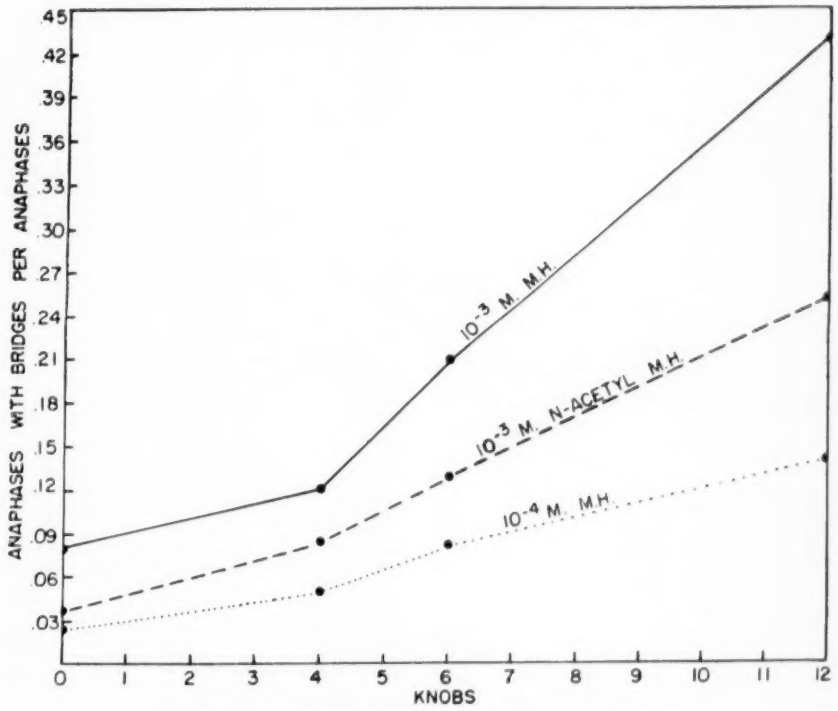


Figure 12

Anaphase bridges induced by MH and N-acetyl MH in four varieties of maize. See text for details.

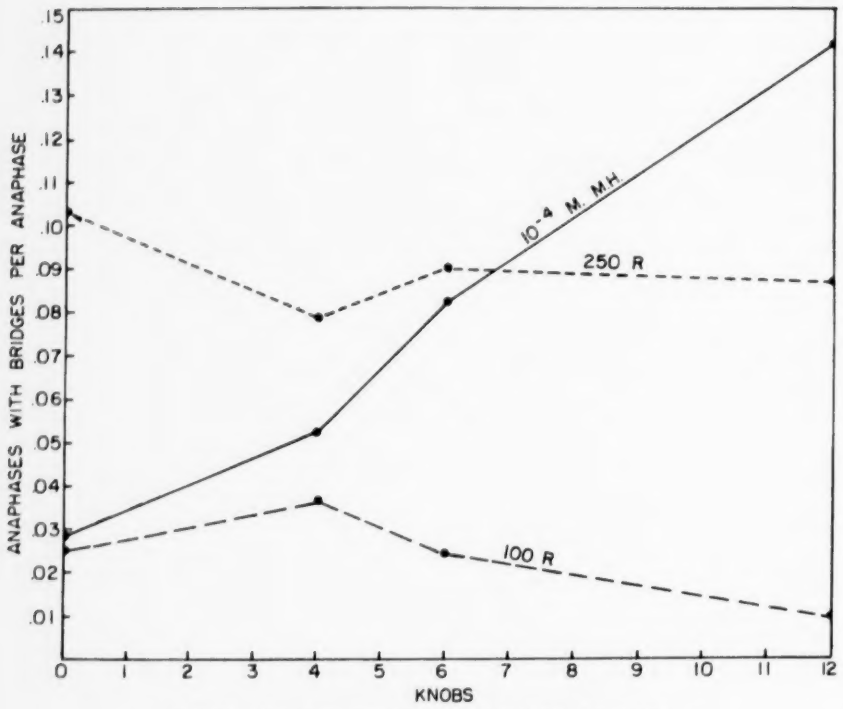
same as cells with single bridges. No discrimination was made with regard to the chromosome or chromatid nature of the bridges. Most cells with bridges also contained readily recognizable chromosome fragments but bridges were scored regardless of whether or not fragments were apparent. The presence of fragments indicates chromosome breakage as opposed to stickiness.

Results

The comparison of X-ray and chemically induced anaphase bridges in the four varieties of corn with different numbers of knobs is shown in Table I. The X-ray treated material does not appear to indicate differential breakage in the varieties tested. On the contrary, MH at two different concentrations, N-acetyl MH and N-benzoyl MH all showed a distinct correlation between number of anaphase bridges and knob number of the maize variety. The results are shown graphically in Figures 12 and 13. In most cases the bridges were roughly five to seven times as frequent in the Zapalote Chico (ZC) as in the knobless flint (KF) for a given chemical treatment.

A comparison of the anaphase bridges induced by MH and MH derivatives is shown in Table II. All of the MH derivatives were found to be capable of causing substantial numbers of anaphase bridges. The activity of the MH derivatives appears to be roughly correlated with the size of the side chain, with a benzenoid group conferring much greater activity than a straight chain group. Indeed, the N-benzoyl MH showed considerably more activity than MH itself.

Persistent nucleoli were found frequently in material treated with MH and MH derivatives but never in controls or X-ray treated material. Aceto-carmine preparations are not ideal for the study of nucleoli but metaphase plates



X-RAY AND MH INDUCED ANAPHASE BRIDGES

Figure 13

Anaphase bridges induced by X-rays and MH in four varieties of maize.

with attached nucleoli were readily recognizable.

Discussion

The high correlation between knob number and bridges would appear to support the hypothesis of Ford[4] and others, that at least some chemicals cause chromosome breakage selectively in heterochromatin. The data do not indicate any obvious relationship between X-ray induced chromosome breakage and knobs. The MH and MH derivatives caused markedly higher bridge frequencies in the 12 knob variety than in the zero, with the four and six intermediate. It should be borne in mind that the size and location of the knobs

TABLE II. Chromosome breakage induced by maleic hydrazide and derivatives in variety Zapalote Chico of maize

Treatment	Anaphases scored	Anaphases with bridges	% Anaphases with bridges
10^{-3} M MH	210	90	42.9
10^{-4} M MH	338	48	14.2
10^{-5} M MH	378	13	3.4
10^{-3} M N-acetyl MH	175	44	25.1
10^{-4} M N-acetyl MH	330	10	3.0
10^{-4} M N-caproyl MH	188	19	10.1
10^{-5} M N-caproyl MH	188	2	1.1
10^{-5} M N-benzoyl MH	179	42	23.5
10^{-6} M N-benzoyl MH	303	11	3.6

were not considered in the present study. These factors combined with sampling deviations would be expected to cause some deviation from linearity in the plot of anaphase bridges against knobs, even if such a linear relationship exists. It would be very unrealistic to assume that the knobless flint has no heterochromatin or that heterochromatin is found only in knobs.

The results with the N substituted MH derivatives are interesting in view of the fact that Loveless[8] found that all of the many structural rearrangements of the MH molecule which he tested had drastically reduced chromosome breaking activity. He did not test any compounds analogous to the MH derivatives reported here. It is by no means inconceivable that the three MH derivatives are all degraded to MH in the corn seedlings. If such is the case, one still must deal with the N-benzoyl MH which showed greater activity than MH itself. There appears to be a negative correlation between the water solubility of the MH derivatives and their chromosome breaking activity. Such a relationship has been found in regard to the c-mitotic activity of a large number of aromatic compounds[7, 12]. This same relationship has also been found for the anti-auxin activity of nitrogen substituted maleimides according to Van Overbeek *et al.*[14].

It should be remembered that in the present work chromosome breakage was measured indirectly as anaphase bridges. The possibility that knobs were acting on reunion rather than on breakage cannot be excluded. This hypothesis would require the assumption that knobs differentially affect reunion in MH and X-ray induced chromosome breaks.

Summary

It has been demonstrated that the number of anaphase bridges induced by maleic hydrazide and certain maleic hydrazide derivatives in corn root tips is highly correlated with the number of heterochromatic knobs in the variety. These results are interpreted as supporting the hypothesis that at least some chemicals cause chromosome breakage selectively in heterochromatic regions. X-ray induced anaphase bridges appeared to be independent of the knob number. All maleic hydrazide derivatives as well as maleic hydrazide itself caused persistent nucleoli, thus the finding of Carlson[2] is confirmed.

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DIPLOID MALE TISSUE AS NEW EVIDENCE ON SEX DETERMINATION IN HONEY BEES

Apis mellifera L.

WALTER C. ROTHENBUHLER*

GENETICS of sex determination in the insect order Hymenoptera is largely unexplored and presents important unsolved problems. Genic-balance theory, based on *Drosophila* and applied widely, does not explain why individuals developing from unfertilized eggs are male, whereas those developing from fertilized eggs are female. The change from the haploid to the diploid chromosome complement does not change genic balance. The first break-through on the problem came as a result of intensive studies on *Habrobracon juglandis* by the Whiting school of researchers (reviewed in Martin[9]). At a single locus, a series of nine alleles has been revealed, and these alleles have been shown to affect sex (Whiting[26]). Female sexual development occurs in individuals of diploid origin that are heterozygous at the sex locus. Male sexual development occurs in all individuals of haploid origin, and in those of diploid origin that are homozygous at the locus in question. Homozygosity for a particular sex allele, in addition to determining maleness, causes death in a high percentage of cases, even though hemizygosity for the same allele, as in a haploid, has no lethal effect.

How widespread this multiple-allele mechanism of sex determination may be, has been given extensive consideration by Whiting [27], White[24,25], and Suomalainen[22]. Speicher and Speicher[21] showed that this mechanism exists also in *Habrobracon brevicornis*. Although *Habrobracon pectinophorae* was reported to possess the multiple-allele sex-determining mechanism[2], *H. pectinophorae* has been found to be the same species as *H. juglandis*[29]. The multiple sex-allele theory without modification cannot explain sex determination in certain parasitic wasps that are highly inbred in nature (Whiting[28], Schmieder and Whiting[20]). Even though the theory is an appealing one as an explanation of sex determination in many cases of haploid male-diploid female phenomena in other orders of the animal kingdom, its experimental demonstration is limited to the Hymenoptera and to two species of one genus therein.

A prominent question, at least among honey-bee geneticists and cytologists, is whether the *Habrobracon* mechanism of sex determination exists in the honey bee, which shows the haploid male and diploid female relationships (Mackensen[5], Sanderson and Hall[18,19], Manning[8], and Kerr and Laidlaw[3]). Mackensen[6,7], over a period of years, has presented data favoring this mechanism. His data indicate that there are multiple alleles, at a given locus in the honey bee, which are lethal in the homozygote, but viable in the heterozygote and hemizygote. Eleven different alleles were shown to exist in six inbred lines[7]. Laidlaw, Gomes, and Kerr[4] estimated that there were 12.4 $\pm$ 3.56 such alleles in one random mating population of 65

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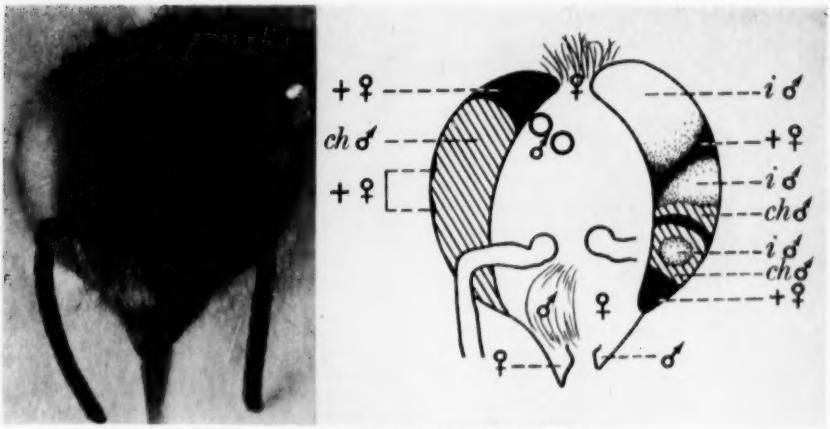


HEAD OF GYNANDROMORPHIC BEE
Figure 14

Photograph and diagram showing color and sex of mosaic patches in eyes as follows: + = wild-type, *ch* = chartreuse, *i* = ivory. Stippling indicates the region of wild-type border effects in which wild-type pigment has developed in the bordering edge of mutant tissue following diffusion of a substance from the wild-type tissue. Other structures of the head are marked as male or female. This individual was a non-exceptional mosaic produced by Mating P—genetic makeup of the mating given in Table I.

colonies of honey bees in Brazil. Diligent searches for genetically marked, phenotypically recognizable, diploid males, expected under the hypothesis, have been carried out. No such male has been reported previous to the present work. From matings which might be expected to produce diploid males, about 50 percent of the progeny die early in development[6], and whether these non-survivors are males has remained an important question. Consequently, in honey bees, it has not been justifiable to refer to sex alleles. Rather, the reference has been to lethal alleles.

It has been expected, since 1948, that gynandromorphism in honey bees would throw light on their sex-determining mechanism (Rothenbuhler, Polhemus, Gowen, and Park[16]). This expectation did not materialize initially except to throw doubt upon Manning's explanation[17]. Instead, it was discovered that male and female parts of a gynandromorph, in the strain of honey bees under consideration, originate as follows: Female parts develop from the zygote resulting from fertilization of the egg pronucleus by a sperm pronucleus; male parts develop from one or more accessory sperms[11,13,14]. These facts were revealed by genetically marking maternal and paternal gametes with mutations for eye color, and identifying the sex and phenotype of eye facets in gynandromorphic progeny. Male facets in most regions of the eye are larger than female facets, and thus recognizable. The difference in size between male and female eyes, which is due, at least in part, to differences in facet size, has been presented pictorially[16]. Figure 14 of the present paper presents a gynandromorph having black female tissue of biparental origin in both eyes, chartreuse tissue of paternal origin in both eyes, and ivory male tissue probably of paternal origin in the individual's left eye. Male tissue of paternal genotype and female tissue of biparental genotype represents the commonly found gynandromorph in this strain. Two types of male tissue, as mentioned elsewhere[12], are found often from matings involving two types of males and producing a high percentage of gynandromorphic progeny. Occasionally, in the many matings that have been under observation, a ge-

netic mosaic that seemed to be completely male or completely female was found. These have always been open to explanation in rather conventional terms involving some sort of binucleate egg.

During the summer of 1956, some matings were found to be producing several mosaics of exceptional phenotype. Each mosaic possessed some wild-type male tissue that was completely unexpected on the bases of the parents involved and known genetics of bees. These mosaics, already discussed briefly at the Tenth International Congress of Entomology in 1956[12] and presented in detail below, are thought to have great significance for the problem of sex determination in bees. The wild-type male tissue seems to be diploid in origin.

Descriptions of Exceptional Individuals

Twenty-two of the exceptional mosaics were produced by Mating P and are presented in Table I; 16 were produced by Mating N and are presented in Table II; and two were produced by Mating O and are presented in Table III. The genetic constitutions of females and males making up the mating are given in each case. Each of the matings produced phenotypically ivory and wild-type workers as expected. Even though equal numbers of each type of drone were used, no particular ratio can be expected in the progeny[23].

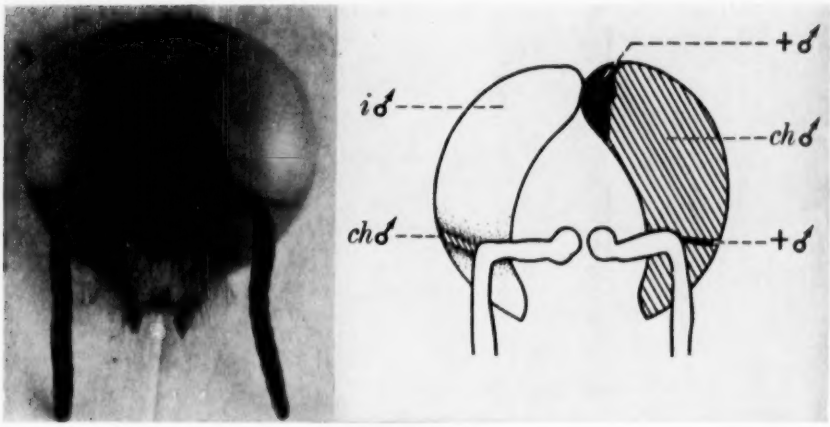
All of the exceptional mosaics emerged in their colonies along with other, more ordinary mosaics, and normal workers and drones. In column one of Tables I, II and III, each mosaic has been given an identifying letter-number designation. The eight individuals constituting the a and b groups were found among 138 other mosaics in a collection of all recognized mosaics on June 18 and 19. The c series of five exceptional mosaics was found among 91 ordinary mosaics on June 23. The d group of five was found among 51 others. The e1 individual was found among 114 other mosaics produced over a period of about three weeks in a small colony to which the mated queen has been introduced. All other mosaics were simply recognized as exceptional and collected during the course of colony inspections.

The frequency of production of exceptional mosaics is of very limited significance in this study for three reasons: What proportion of exceptional wild-type (diploid?) male tissue can survive in mosaic combination with other tissue is unknown; several drones were used to inseminate each queen making the percentage of progeny fathered by each drone un-

TABLE I. Brief descriptions of the exceptional mosaics from Mating P: ♂ *i*/+; *ch*$^{+}$/ ♂♂ from Mating Z ♀ *i*/*i*; *ch*$^{+}$//*ch*$^{+}$♀ × ♂ *i*$^{+}$/+; *ch*/ ♂♂ from Mating Q ♀

Designation of individual	Eye phenotypes and their estimated extents *i* ♂	*ch* ♂	+ ♂	Border interactions	Relative size of ♂ facets
a1		1/2	1/2		
a2	1/2		1/2		+ larger
a3	1/8		7/8		+ larger
b1		5/8	3/8		+ larger
b2	1/4		3/4		?
b3			1*		
b4		5/8	3/8		
b5	4/8	1/8	3/8		+ larger
c1	*	5/8	3/8	*	?
c2	1/2		1/2	+ in *i*	+ larger
c3	1/8*		7/8	+ in *i*	+ larger
c4	4/8	1/8	3/8	+ in *i*	+ larger
c5		1/8	7/8		
d1	4/8	3/8	1/8	+ in *i* at *i-ch* junction	+ larger
d2		3/8	5/8		+ larger
d3		7/8	1/8		+ larger
d4		5/8	3/8		?
d5		1/2	1/2		
e1		1/8	7/8		
ee1	7/8		1/8	+ in *i*	?
ee2	2/8	3/8	3/8	+ in *i* at *i-ch* junction	+ larger
ee3	3/8	3/8	2/8	+ in *i*	+ larger

*See text for further information.



MOSAIC MALE
Figure 15

Individual designated as dl in Table I. Labeling same as in Figure 14. Stippling indicates region of wild-type border effects, but in this case the diffusable substance has come from chartreuse tissue.

knowable; and exceptional tissue is detectable only in the eyes. The important questions are: What is the exact appearance of the specimens? Were they, in fact, produced by the mated queens heading the colonies? And how can they be interpreted?

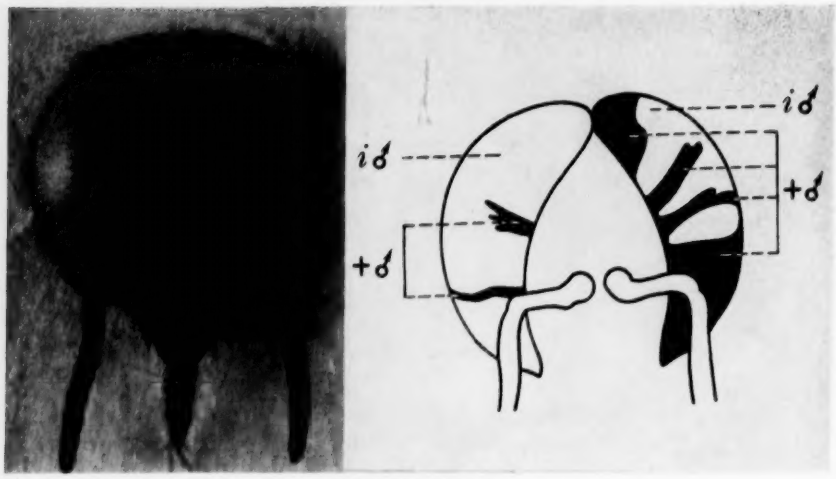
A picture of individual dl is presented in Figure 15 which will help in the explanation of the tabular columns. The mosaic's right eye (left side of picture) is seen to be almost all ivory and completely male. The left eye is largely chartreuse and completely male. In the left eye are two patches of wild-type, male tissue—one at the top and the other about three-fourths of the way down. In denominations of eighths, and to the nearest eighth, the individual is estimated to have four-eighths of its eye tissue ivory, three-eighths chartreuse, and one-eighth wild-type (over-estimate in this case due to the fractional denomination used throughout the table) and these fractions are entered in columns two, three and four.

It is not clear in the photograph, but the right eye has a very narrow band of chartreuse tissue near the angle of the right antenna. As is expected in mosaic combinations of char-

TABLE II. Brief descriptions of the exceptional mosaics from Mating N:
***i*//*i*; *ch*⁺//*ch*⁺♀ × 4 *i*//*i*; *ch*//*ch* ♂♂ from Mating Q ♀**
4 *i*⁺//*i*; *ch*//*ch* ♂♂ from Mating Q ♀

Designation of individual	Eye phenotypes and their estimated extents			Border interactions	Relative size of ♂ facets
	i ♂	*ch* ♂	+ ♂		
f1	7/8		1/8	no + in *i*	+ larger
f2	5/8		3/8	no + in *i*	?
f3		7/8	1/8		+ larger
f4		1/2	1/2		
f5		3/8	5/8		+ larger
f6		3/8	5/8		?
g1	3/8	4/8	1/8	no + in *i*	+ larger
g2	4/8	1/8	3/8	no + in *i*	+ larger
g3		3/4	1/4		+ larger
h1*	7/8		1/8	no + in *i*	?
h2*	5/8		3/8	no + in *i*	+ larger
h3	3/4		1/4	no + in *i*	?
h4		3/4	1/4		+ larger
i1	5/8		3/8	no + in *i*	+ larger
i2	5/8		3/8	no + in *i*	+ larger
i3	5/8	1/8	2/8	no + in *i*	+ larger

*See text for further information.



MOSAIC MALE
Figure 16

Individual designated il. Labeling as in Figure 14 and 15. No wild-type border effects are observed.

treuse and ivory tissue[15], wild-type pigment has formed in the bordering facets of the ivory tissue. Wild-type and ivory mosaic combinations also lead to wild-type pigment production in the edge of the ivory tissue. No such border effects occur in chartreuse tissue mosaic with wild-type. Presence of wild-type pigment in ivory tissue is indicated by + *in i* in the fifth column, and its converse by prefixing the word *no* (which will be discussed presently). In the case of dl, it is indicated that there is wild-type pigment in the border of the ivory tissue at the junction of the ivory and chartreuse tissues.

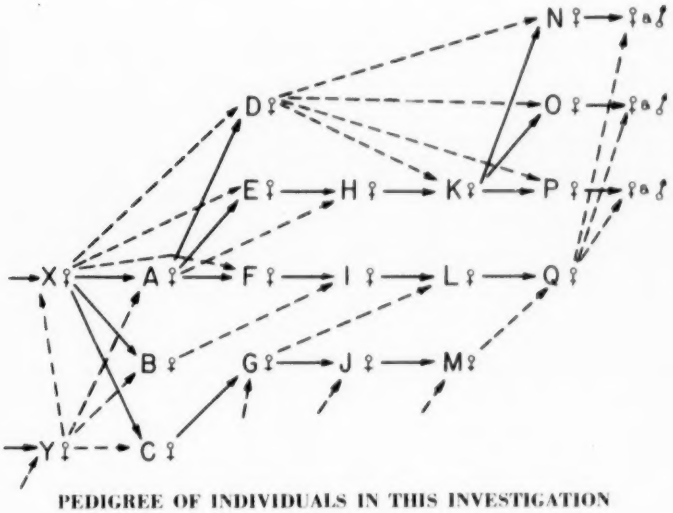
Figure 16 presents individual il, which has *no* + *in i* border effects. This individual was produced by a different mating, Mating N, in which the ivory drones were also mutant for chartreuse. The ivory male tissue has developed from an accessory sperm, and wild-type pigment cannot develop in *i/; ch/* tissue. Diffusion can repair the deficiency in ivory tissue but it cannot repair that in chartreuse tissue. As expected, on the basis of physiological genetics, chartreuse pigment appeared to be present in the border of this *i/; ch/* tissue. Chartreuse pigment is sometimes difficult to see in border effects and the amount seems to vary from case to case. At any rate, the primary purpose of border-effect observations in Mating N progeny is to learn whether the ivory tissue actually developed from a sperm rather than an egg nucleus. Presence or absence of wild-type pigment is sufficient to answer this question, and consequently, is the only observation recorded herein.

In certain cases and for various reasons, no observation on border effects has been entered. The al bee, for instance, has one eye entirely chartreuse and one entirely black permitting no observation. Even if one eye had been mosaic, no observation would have been recorded because, as already indicated, an effect of wild-type tissue on chartreuse never has been seen and presumably does not occur. In a few of the earlier cases, observations on border effects were not recorded.

It was noticed in the examination of the first few exceptional mosaics that the wild-type male facets seemed larger than their neighboring chartreuse or ivory male facets. Cursory measurements on individuals a2 and a3 con-

TABLE III. Brief descriptions of the exceptional mosaics from Mating O:
i//i; ch⁺//ch⁺ **♀ × 3** ***i⁺/; ch/*** **♂♂ from Mating Z ♀**
i//i; ch⁺//ch⁺ **♀ × 3** ***i⁺/; ch/*** **♂♂ from Mating Q ♀**

Designation of individual	Eye phenotypes and their estimated extents			Border interactions	Relative size of ♂ facets
	i ♂	*ch* ♂	+ ♂		
j1	3/8		5/8	+ in *i*	+ larger
j2	3/8		5/8	+ in *i*	+ larger



PEDIGREE OF INDIVIDUALS IN THIS INVESTIGATION

Figure 17

Arrows represent gametes—solid arrows = eggs, broken arrows = sperms. Since drones develop from unfertilized eggs, each one is a gamete from a queen and needs gametic representation only in a pedigree. This pedigree shows that the N, O, and P queens are related to the queen's drones with which they were mated.

firmed the superficial appearance and indicated that the wild-type facets measured had approximately one-eighth greater diameter than the adjoining mutant ones. Thereafter if wild-type tissue appeared to have larger facets, the fact is indicated in the table by + *larger* in the sixth column. Figure 15 (individual d1) presents an individual with larger wild-type facets. It was not always possible to see a clear difference in mutant and wild-type facet size, and such instances have been recorded with a question mark. In no case did wild-type facets ever appear to be smaller than mutant facets. In a few cases, there was not a suitable distribution of tissues to permit comparison of facet sizes at a border and, in a case or two, the observation was simply omitted.

The tabular presentation does not give sufficient information on certain specimens. They have been marked to draw attention to this fact and are now discussed in order.

Mosaic b3 had misshaped eyes. They seemed to be male and definitely were wild-type, but why they were misshaped is not known.

Mosaic c1 had a very small patch of tissue, which was probably ivory, in the bottom of the chartreuse, male eye. Sex of the supposed ivory tissue was impossible to determine, because there is a small region near the bottom of bees' eyes where size of male and female facets are identical. Wild-type pigment was present in its border.

Mosaic c3 had ¼ of one eye (⅛ of its eye tissue) ivory. The ivory tissue was at the bottom of the eye and was male in conformation. Nevertheless, the facet size on the ivory side of the border was so much smaller than on the wild-type side that one wonders whether the ivory tissue might be female. On the other hand, this general impression of size discrepancy could be brought about by over-size wild-type male facets.

Mosaics h1 and h2 showed female tissue. Part of one hind tibia of h1 and part of the last, visible, ventral abdominal plate of h2 was female. This was the only clearly identifiable female tissue in the entire series of individuals.

Mosaicism appeared in the abdomens of a high proportion of mosaics. Of 25 mosaics examined for abdominal mosaicism, 17 showed it, suggesting the possibility that breeding tests might reveal gonadal mosaicism.

Figure 17 presents the pedigree of the queens and drones involved in the production of the mosaics in question. Absence of males in bee pedigrees is not new[10]. Each broken line from a queen represents one of her gametes which developed into a drone (used in an insemination) which provided, in turn, the spermatic contribution to the indicated zygote. In the interest of clear understanding, it is worth emphasizing that the Mating-Q *queen* produced the drones and Mating Q itself (which includes the drones with which the Mating-Q queen was inseminated) is of no present interest. The Mating-Z queen (mentioned in Tables I and III) was a sister of the Mating-L queen and although her ivory drones figured in Matings P and O, their presence in the

pedigree would add unnecessary detail. These ivory drones did not father the exceptional tissue. The critical item is the common ancestry of the Q queen with the N, O, and P queens, because an attempt will be made to show that drones from the Q queen did father the exceptional male tissue.

Further Information, Discussion and Interpretation

Were the exceptional mosaics under consideration produced by the supposed matings? This is a question of critical importance. The possibility that these individuals drifted into the colonies where they were found, and the possibility that they were produced by laying workers must be considered.

Drifting seems extremely unlikely for several reasons. In the first place, there were no matings in our apiaries which could produce such mosaics by any readily conceivable process other than the one to be advanced later. Matings that would be under suspicion in this connection would include queens heterozygous for both ivory and chartreuse (*i*//*i*+ *ch*//*ch*+), and ivory queens mated simultaneously to chartreuse and wild-type drones. Secondly, the colony containing Mating P was shaken out of its hive and made to re-enter through a queen excluder on June 11. This process excludes all normal-sized drones and most or all smaller drones reared in worker cells. This excluder was left in place thereafter, and most or all of the mosaics collected were of such size that they could not have entered through the excluder. Thirdly, the entire colony was moved on June 14 to another yard two miles distant and located approximately 100 yards from other bees in the same general location. Thereafter, repeated collections of all mosaics were made and drifting seems an impossibility. Matings N and O were located in the yard from which Mating P was moved. So exceptional mosaics were found in colonies located in two different apiaries.

The possibility that worker bees laid unfertilized eggs which developed into the exceptional mosaics seems unlikely. Here again Mating P provides critical evidence. Part of the worker bees (diploid females) produced by this mating would be heterozygous at both the ivory and chartreuse loci. Conceivably oogenesis and subsequent development of an egg from such a worker could give rise to one of these complex mosaics. Usually, eggs from such workers would be expected to produce ivory, chartreuse, and wild-type drones in a 2:1:1 ratio. Inasmuch as no wild-type drone was seen in the colony (other than the possibility that b3 was such), egg laying activity by workers was not very great.

To further eliminate the possibility of laying workers confusing the results, the Mating P queen was removed from her colony and introduced to a nucleus hive, made up of wild-type bees from an outyard, and located in some bushes approximately 75 yards from the parent colony. Drifting between these two units was quite unlikely due to their surroundings as well as distance of separation. Mosaic el was produced in this nucleus hive where all progenies of laying workers, if they occurred, were expected to have only wild-type eyes. It seems certain that Mating P, and not laying workers, was producing the mosaics found in its hive.

A last remaining question would inquire about the possibility of a mistake in specifying the genetic constitutions of the queens and drones making up the matings. All three queens were ivory-eyed, indicating homozygosity for the recessive ivory-eye mutant. Furthermore, the Mating-P queen's genetic constitution was tested by examining a sample of her gametes as manifested in 382 drones. The colony was given a drone comb in which the queen could oviposit. Before emergence of the drones, the comb was taken into a warm-room away from other bees. Every drone, of 382 observed, had ivory eyes and appeared completely normal in every way. The Mating-P queen, as well as the others, surely was homozygous for ivory eyes. Likewise, there is no evidence of any mistake relative to the drones used, and there seems to be no possibility that a wild-type drone could have been used by mistake in each of three different inseminations.

In light of the above considerations, it seems certain that Matings N, O, and P, were of the indicated genotypes and actually produced the mosaics in question. Since no single gamete of the parents involved could have given rise to the wild-type male tissue, it is suggested that this tissue arose from the union of two gametes—an egg (*i*/; *ch*+/) and sperm (*i*+/; *ch*/)—and is diploid in origin. The accompanying ivory and/or chartreuse male tissue arose from accessory sperms. Larger facet size in the wild-type tissue than in the mutant tissue supports this interpretation Grosch[1] showed that diploid male *Habrobracon* had larger facets than haploid males. Mutation or complex interactions of tissue are not considered to be adequate explanations because they leave facet size, high frequency of occurrence, and other observations unexplained.

The only difference between these mosaics and the gynandromorphs commonly found is that the zygote (resulting from union of egg and sperm pronuclei) which commonly develops into female tissue has, in the case of these mosaics, developed into male tissue. The origin of the small amount of female tissue seen in two of the 40 mosaics is obscure. Among various possibilities for its origin are binucleate eggs with fertilization producing two zygotes or the union of two sperms to produce a second zygote.

Kerr and Laidlaw[3] reviewed several hypotheses of sex determination in bees which may be examined anew in the presence of diploid male tissue. The one which they designated a mere description of the facts, haploidy of males and diploidy of females, no longer in-

cludes all observations. Likewise, a balance of maleness in cytoplasm versus femaleness in chromosomes seems inadequate in the face of diploid male tissue. The hypothesis of genes for femaleness having cumulative effect and other genes for maleness having non-cumulative effect requires further modification or new assumptions to meet the new observations. Only the sex-allele hypothesis among those already formulated actually predicts the present occurrence.

It has been shown that a queen bee having a "lethal" allele in common with the drone to which she is mated ($X^a//X^b$ by $X^a/$) produces about one-half inviable eggs ($X^a//X^a$). If these lethal alleles are involved in sex determination, as they are in *Habrobracon*, a few lethal-allele homozygotes might be expected to survive and undergo male differentiation. No such individual has been recognized, however, until the presently studied mosaics in which male tissue of diploid origin was enabled to survive only by virtue of its association with normally viable haploid male tissue. It is supposed that the queens involved in Matings N, O, and P had a lethal allele in common with at least one chartreuse drone in each case. The tissue resulting from syngamy of gametes with identical sex alleles, when it survived, underwent male development. The lethal alleles, therefore, are actually sex alleles.

Further experimentation involving single drone matings are in progress.

Conclusion

Inviability of a proportion of the eggs from matings of closely related individuals reported by others, and the present report of diploid male tissue in mosaics from related parents constitute strong evidence for the sex-allele hypothesis of sex determination in honey bees. This mechanism of sex determination now seems to exist in at least two species of the genus *Habrobracon* (family Braconidae) and one species of the genus *Apis* (family Apidae).

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Biochemical Individuality

THIS book* is one of the results of Dr. William's many years of biochemical research and of his long-standing interest in individual variation. Essentially it is an essay in which presentation of the thesis, biochemical variation, is followed by a discussion of its genetic basis, evidence for its existence, and its implications. A great deal of evidence for individual variation has been gathered from many sources, including the author's own works. There are separate chapters devoted to variation in anatomical features, in composition, enzymic patterns, endocrine activity, excretion patterns, drug sensitivity, nutrition, and a chapter on miscellaneous variation. Although some of the evidence is "far from being as satisfactory as it would have been had the investigations cited been interested in the problem of individuality," and some studies directly relating to the problem have been omitted, the range and variety of data is impressive and stimulating.

The genetotrophic concept is described as follows. "Every individual organism that has a distinctive genetic background has distinctive nutritional needs which must be met for optional well being." According to this view, a large variety of diseases may be caused primarily by the failure of the individual to meet his peculiar nutritional needs. As Williams emphasizes, the importance or non-importance of this concept, depends on how great the individual variability is. Thus, precise evaluation of the importance of the genetotrophic concept must await basic research in this area by geneticists and biochemists.

The last three chapters of the book are devoted to a discussion of the implications of biochemical individuality and the genetotrophic concept for the biological sciences and for research in medicine, dentistry and psychiatry. The value of an understanding of individual variation to physicians and dentists engaged in practice or research is well emphasized.

Although the book should be of interest to the geneticist, most of the ideas expressed will not be new. Many biochemists, on the other hand, may not be aware of some of the concepts and material presented. If some of these are stimulated to work in this area, the book will have served a doubly important function.

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**Biochemical Individuality. The Basis for the Genetotrophic Concept.* Roger J. Williams. John Wiley & Sons, Inc. pp. 214, 17 figures, 17 tables. 1956. $5.75.

INTERACTION OF GENES FOR ROUND LEAF AND FREGO BRACT IN COTTON

C. F. Lewis*

INTEREST in the cytogenetics of the genus *Gossypium* makes it important to study systematically the linkage relationships of known marker genes in cotton. Stephens[4] published evidence for a large number of independent segregations and four linkage groups; however, the task of determining the linkage relationships of all known cotton genes has by no means been completed. Brown and Cotton[1] and Richmond and Harper[3] found that a leaf abnormality known as round leaf was inherited as a recessive. Green[2] described another recessive mutant called frego bract. The original purpose of the present study was to determine the linkage relationship between these two genes, but as the work progressed it became necessary to explain a peculiar type of interaction which was apparent in the segregating material.

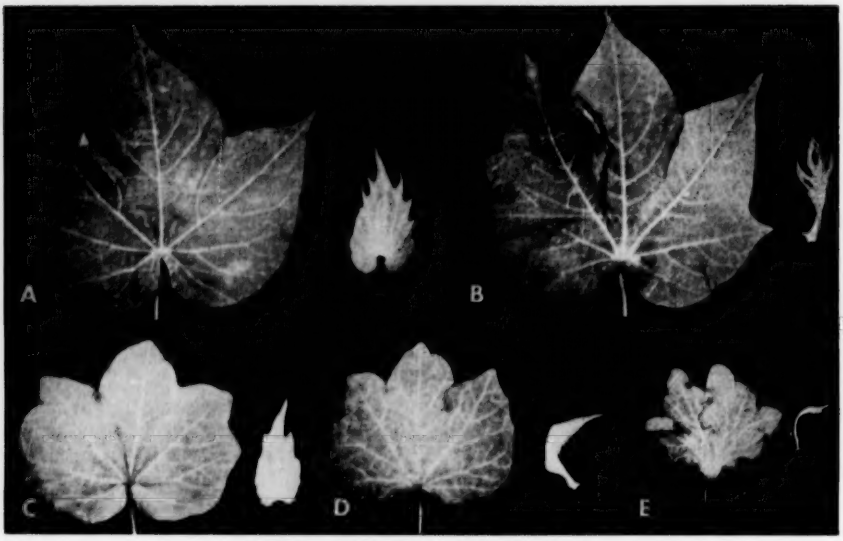
Materials and Preliminary Results

Genetic stocks of round leaf and frego bract, maintained under Regional Research Project S-1, Genetics and Cytology of Cotton, were used as parental material. Stephens[4] mentioned that crenate (*cn*) was sometimes called round leaf, and a test for homology by the author showed that the genes for crenate and round leaf are the same. A cross was made between round leaf, non-frego bract and non-round leaf, frego bract. The F_1 plants were non-round leaf, non-frego bract as expected of recessive genes. A plant with a round leaf, frego bract phenotype was selected as a tester stock from a small F_2 population. Accordingly testcrosses of (F_1 round, non-frego $\times$ non-round, frego) $\times$ round, frego were made. In an F_2 and two testcross progenies a phenotype with an extreme expression of both round leaf and frego bract appeared. Furthermore, as shown in Table I, the parental combinations in both testcrosses were fewer than expected on the basis of normal dihybrid segregation. At this point a progeny test of the round leaf, frego bract tester stock, which had been assumed to be a double recessive, was made. Eleven greenhouse plants segregated into 5 round leaf, frego bract, 3 round leaf, non-frego bract, and 3 extreme round leaf, extreme frego bract. These 11 greenhouse plants were progeny tested in the field the following year. The three extreme round, extreme frego plants and the three round, non-frego plants bred true in populations of 98 and 42 plants respectively. The pooled classification from the five round, frego plants was 20 round, non-frego, 36 round frego, and 19 extreme round, extreme frego which fits a theoretical 1:2:1 ratio, $\chi^2 = .15$, $P = .50 - .95$. It was apparent that plants with the round leaf, frego bract phenotype were homozygous for round leaf but heterozygous for frego bract, and that extreme round leaf, extreme frego bract was the true double recessive. The testcross results in Table I then could be explained on the assumption that the round, frego tester stock was homozygous round but heterozygous frego. The F_2 data in Table II could be explained on the basis that the frego phenotype is conditioned by a single heterozygous locus in the presence of homozygous round leaf. The actual classification of the F_2 did not differ significantly from the 9:3:1:2:1 theoretical ratio.

Genetic Hypothesis and Final Results

The genetic hypothesis based on the preliminary data is expressed in the following theoretical genotypic-phenotypic relationship

*Contribution from the Cotton Section, Field Crops Research Branch, Agricultural Research Service, U. S. Department of Agriculture in cooperation with the Department of Agronomy, Texas Agricultural Experiment Station, College Station, Texas. A part of this work was done under Project S-1 of the Hatch Act (amended).



ROUND LEAF AND FREGO BRACT
Figure 18

Genotypic-phenotypic relationship in a cross involving genes for round leaf (*rl*) and frego bract (*fg*) in cotton. *A—Rl-Fg-*, Non-round, non-frego; *B—Rl-fgfg*, Non-round, frego; *C—rlrl FgFg*, Round, non-frego; *D—rlrl Fgfg*, Round, frego; *E—rlrl fgfg*, Extreme round, extreme frego.

in a dihybrid ratio of a cross involving genes for round leaf (*rl*) and frego bract (*fg*).

Ratio	*Genotype*	*Phenotype*
1	*RlRlFgFg*	non-round, non-frego
2	*RlrlFgFg*	non-round, non-frego
2	*RlRlFgfg*	non-round, non-frego
4	*RlrlFgfg*	non-round, non-frego
1	*RlRlfgfg*	non-round, frego
2	*Rlrlfgfg*	non-round, frego
1	*rlrlFgFg*	round, non-frego
2	*rlrlFgfg*	round, frego
1	*rlrlfgfg*	extreme round, extreme frego

This genotypic-phenotypic relationship differs from a typical dihybrid in two respects. (1) The two mutants interact to give an extreme expression of both characters when they are homozygous in the same plant. (2) On a homozygous round leaf background, the gene for frego bract undergoes a change in dominance. Photographs of the five phenotypic classes are shown in Figure 18.

In the final study, critical evidence for the genetic hypothesis was obtained from the following populations of plants:

1. *RlrlFgfg* × *rlrlfgfg*, (F_1 extreme round, extreme frego × non-round, non-frego) × extreme round, extreme frego. This was a testcross in coupling phase and assuming independent assortment would be expected to produce a 1:1:1:1 ratio of non-round, non-frego; non-round, frego; round, frego; extreme round, extreme frego.

TABLE I. Number of plants in each phenotypic class and analysis of the testcrosses *RlrlFgfg* × *rlrlfgfg*, (F_1 round leaf, non-frego bract × non-round leaf, frego bract) × round leaf, frego bract

Year	Non-round Non-frego *Rl—Fg—*	Non-round Frego *Rlrlfgfg*	Round Non-frego *rlrlFgfg*	Round Frego *rlrlFgfg*	Extreme round, Extreme frego *rlrlfgfg*	Total Plants
1954	27	12	10	25	9	83
1955	68	14	14	32	21	149
Total	95	26	24	57	30	232
Exp.	87	29	29	58	29	232

χ^2 (3:1:1:2:1) = 1.96 P = .50 - .95

2. *RlrlFgfg* × *rlrlfgfg*, (F_1 round, non-frego × non-round, frego) × extreme round, extreme frego. This was a test-cross in repulsion phase and would be expected to give the same results as population 1.
3. *rlrlFgFg* × *RlrlFgfg*—Round, non-frego × (F_1 round, non-frego × non-round frego). The theoretical ratio from this cross is *2* non-round, non-frego; 1 round, non-frego and 1 round, frego.
4. *rlrlFgFg* × *rlrlfgfg*—Round, non-frego × extreme round, extreme frego.

The results from populations 1, *2* and *3* are given in Tables III, IV and V, respectively. The data in all cases agree with the hypothesis based on the preliminary data and indicate that the genes for round leaf and frego bract are not linked.

A cross of *rlrlFgFg* × *rlrlfgfg* (round, non-frego × extreme round, extreme frego) gave a uniform progeny of 15 round, frego plants. Additional progeny tests of F_2 plants confirmed the results obtained in the preliminary data that extreme round, extreme frego and round non-frego plants breed true, but round, frego plants segregate into a 1:2:1 ratio of round, non-frego; round, frego; and extreme round, extreme frego, respectively.

Discussion and Summary

Mutant genes for round leaf and frego bract act as recessives when studied separately. The gene for round leaf, however, causes the bract to be smaller and less toothed than a normal bract as illustrated in Figure 18. The gene for frego bract produces a narrow, twisted bract.

TABLE II. Number of plants in each phenotypic class and analysis of the F_2 round leaf, non-frego bract × non-round leaf, frego bract

	Non-round Non-frego *Rl—Fg—*	Non-round Frego *Rl—fgfg*	Round Non-frego *rlrlFgFg*	Round Frego *rlrlFgfg*	Extreme round, Extreme frego *rlrlfgfg*	Total Plants
Obs.	50	15	9	8	6	88
Exp.	49.5	16.5	5.5	11.0	5.5	88

χ^2 (9:3:1:2:1) = 3.23 P = .50 - .95

TABLE III. Number of plants in each phenotypic class and analysis of the testcross *RlrlFgfg* × *rlrlfgfg*, coupling phase

	Non-round Non-frego *RlrlFgfg*	Non-round Non-frego *Rlrlfgfg*	Round Frego *rlrlFgfg*	Extreme round Extreme frego *rlrlfgfg*	Total Plants
Obs.	61	51	56	51	219
Exp.	54.75	54.75	54.75	54.75	219

χ^2 (1:1:1:1) = 1.25 P = .50 - .95

TABLE IV. Number of plants in each phenotypic class and analysis of the testcross *RlrlFgfg* × *rlrlfgfg*, repulsion phase

	Non-round Non-frego *RlrlFgfg*	Non-round Non-frego *Rlrlfgfg*	Round Frego *rlrlFgfg*	Extreme round Extreme frego *rlrlfgfg*	Total Plants
Obs.	52	61	65	58	236
Exp.	59	59	59	59	236

χ^2 (1:1:1:1) = 1.52 P = .50 - .95

TABLE V. Number of plants in each phenotypic class and analysis of the cross *rlrlFgFg* × *RlrlFgfg*

Population	Non-round Non-frego *RlrlFgfg*	Round Non-frego *rlrlFgFg*	Round *Frego* *rlrlFgfg*	Total Plants
Obs.	65	39	27	131
Exp.	65.50	32.75	32.75	131

χ^2 (2:1:1) = 2.21 P = .30 - .50

When these two mutants are heterozygous in the same plant, both genes act as recessives and the plant has a normal leaf and bract. When a plant is homozygous for round leaf, the smaller bract associated with the round leaf gene can be narrowed and twisted into the frego bract phenotype by a heterozygous frego genotype. This interaction between the two genes amounts to a loss of dominance of the non-frego bract gene on a genetic background homozygous for round leaf. Further, when both mutants are homozygous the leaf and bract are extremely abnormal as illustrated in Figure 18. These results indicate that caution must be taken in scoring recessive mutants in cotton especially when the mutants are in different genetic backgrounds as in cytogenetic studies involving amphidiploid and diploid species.

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CONGENITAL LOCO IN TURKEYS

R. K. Cole*

An inherited lethal condition identical with that known in the domestic fowl as congenital loco, described by Knowlton[3] and subsequently assigned the gene symbol *lo* by Hutt[2], has been recognized in the Empire strain of broad-breasted white turkeys. The use of *lo* for congenital loco in turkeys is suggested so as to maintain uniformity of symbols for identical characters in different species. Affected poults, which are easily recognized at hatching, show all the characteristics of congenital loco in the fowl but their movements are perhaps a little more extreme or violent (Figures 19 and 20). Although affected poults hatch in excellent physical condition they have not lived longer than a week even when fed and watered by hand. The character is therefore an obligate lethal.

The genetic basis for the condition was certain when the data for three matings in pen J for 1955, involving one sire and three closely related dams were summarized (Table I). The parents were entirely normal but produced 32 loco poults among 122 progeny. Since this represented a hatchability of over 85 percent of fertile eggs with slightly more than 25 percent showing the lethal, it is clear that the gene is injurious only after hatching, as was found by Knowlton to be the case in the fowl. The first common ancestor in the pedigree of these poults was a hen hatched in 1951. There were other common ancestors so that the origin of the lethal gene cannot to be definitely verified.

Since the breeders used in pen J of 1955 were considered outstanding because of body type and excellent reproduction, the desire to keep the line but to eliminate *lo* was such as to warrant a testing of normal survivors from those matings.

The results of these and other tests fully confirmed the original interpretation and indicated that the lethal gene was far more widely distributed in the flock than had been thought.

Using the one male and the three females, previously identified as heterozygous, tests with artificial insemination were made for the genotype of certain survivors from the J-pen matings. With the first hatch in February, 1956, a young tom was identified as a carrier. Three of the four young hens mated to their sire (J sire of 1955) also proved to be heterozygous for congenital loco. Since a single insemination in the turkey can result in fertility for a period of up to eight weeks, considerable time between tests had to elapse to ensure return of infertility in the females. However, during the season some hens were used to test as many as three sires, while a single known carrier sire would be used on several females. Thus it was possible to identify potential breeders that were *Lo Lo* with which the breeding of the strain could be continued.

Other matings, using chiefly the progeny of two outstanding sires, soon revealed the presence of *lo* in other individuals and that the sires for pen A and probably also pen H, like that for pen J, had been carriers of loco. In fact, of the nine single-sire matings of 1955 from which progeny were retained, and hence available for testing, two and possibly three were headed by heterozygous sires, and at least three other pens included one or more carrier females. Of the 71 families from which progeny were used in 1956, no less than 25 included individuals that were heterozygous for congenital loco. It thus became desirable to test for *lo* all of the sires used in 1956. The com-

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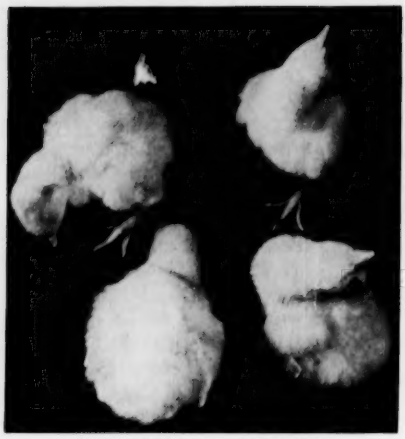

LOCO POULTS AT REST
Figure 19
The affected poult holds its head resting on the back or supports it by touching the beak to the floor.

LOCO POULTS REACTING TO STIMULATION
Figure 20
The presence of a noise or vibration causes the affected poults to lose their balance. They regain the position shown in Figure 19 only with difficulty.

bined results of these tests are also presented in Table I.

Barring errors in pedigree, the production of one or more loco poults identifies both parents as heterozygous. Because of chance and the expected 3:1 ratio, small families might not reveal the true genotype of the bird under test. However, with 11 or 16 progeny, all normal, the probability that the tested parent was heterozygous would be $< .05$ and $< .10$, respectively. Since individuals with fewer than 11 normal progeny are not considered proven to be *Lo Lo* the families from three sires with 7 to 9 normal poults are not included in the tabulated data. Of the 37 individuals considered as heterozygous, *Lo lo*, all have produced at least three loco poults, except a female with two and a sire with a single lethal poult.

Among the birds tested there were three families in which two-thirds of the phenotypically normal birds should have been heterozygous and other families in which only one-half should have been carriers. Among the birds adequately tested (33) close agreement was found between observed and calculated ratios (Table II).

In a mating in 1956 consisting of 10 hens, including nine full or half-sisters from a carrier sire, half of which should be expected to be heterozygous, and a tom which proved to be a carrier, there were loco poults in all progenies. Since

TABLE I. Incidence of congenital loco in turkeys from matings of known genotypes

Year	Source of data	Genotype of Sires	Genotype of Dams	Matings no.	Eggs no.	Poults no.	Loco %	Hatch. %
1955	Pen J	*Lo lo*	*Lo lo*	8	143	122	26.2	85.3
1956	Pen H	*Lo lo*	*Lo lo*	10	436	261	21.8	60.0
1956	Testing	*Lo lo*	*Lo lo*	23	516	405	27.2	78.5
Totals:		*Lo lo*	*Lo lo*	36	1095	788	25.3	72.0
1956	Testing	*Lo lo*	*LoLo*	9	236	190	0	80.5
		LoLo	*Lo lo*	11	256	207	0	80.9

all of the turkeys kept for breeding had been previously selected for body conformation and viability, it seems certain that heterozygosity for the gene *lo* was not detrimental to them.

Discussion

There are many instances of similar genetic characters in different species of animals, such as albinism, pigment type and distribution in hair or feathers, and even sex-linked hemophilia in man and dog. The occurrence of a lethal with such a clear cut syndrome as congenital loco in economically important and domesticated representatives from two families within the order Galliformes is of more than passing interest. The anatomical basis for the defect is not known but appears to center in the mechanism for maintaining spacial equilibrium. Gross and microscopic examination of the brains from loco poults have been entirely negative.

Howell[1] summarized the work of Fluorens and others, who studied the semicircular canals of pigeons: "When all three canals are cut on one or both sides the animal shows a distressing inability to maintain a normal position. The head is twisted, it is not able to stand unless supported, and any attempt at walking or flying results in violent and incoordinated movements. It should be added that results of this character are obtained only when the membranous canals are injured." A study of the semicircular canals and their ampullae might reveal the anatomical basis for congenital loco.

There is some evidence that congenital loco was present in an earlier generation of this stock of turkeys. However, because of efforts to prevent close breeding, its appearance in frequencies sufficient to indicate a genetic lethal did not occur until the gene was widespread among the turkeys selected for breeding purposes. From birds mated or tested for the gene in 1956 more than 20 percent proved to be heterozygous. About 220 hens were mated, but some had too few progeny to prove the dam's genotype, and others were mated only with males homozygous for the normal allele. However the frequency of *lo* in the breeding flock was not less than .1 and probably somewhat higher.

As a consequence of the testing procedure used in 1956 and its continuance early in 1957 on some individuals not fully proven to be free of the lethal gene, along with the use again of certain older breeders known to be *Lo Lo*, the turkeys used to perpetuate the stock this year are free of the lethal gene and should continue so, barring a new mutation.

Summary

Congenital loco in turkeys is an obligate postnatal lethal caused by the homozygous state of the recessive gene *lo*. Birds heterozygous for this gene are fully viable and show no deviations from normal performance.

The lethal is identical in all known respects to the one initially described in the fowl by Knowlton[3] nearly 30 years ago. By testing with known heterozygous breeders it has been possible in one season to eliminate the gene from a breeding flock in which its frequency had reached a minimum of 0.1.

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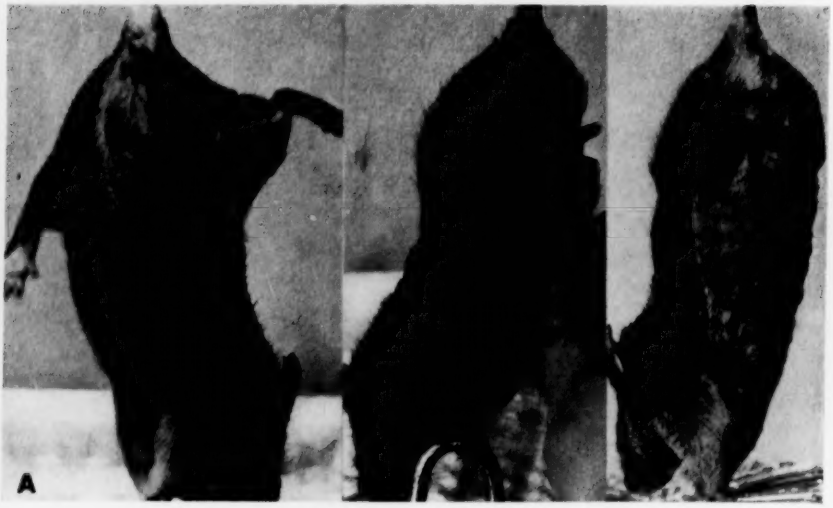
TABLE II. Ratios of carriers to non-carriers of congenital loco in normal progeny from two types of matings

Heterozygotes expected, frequency	Birds tested no.	Heterozygotes	
		Observed no.	Calculated no.
2/3	18	14	12
1/2	15	7	7½
	33	21	19½

QUIVERING, A NEW FIRST CHROMOSOME MUTATION IN MICE

C. H. Yoon and E. P. Les*



NORMAL AND QUIVERING

Figure 21

A—A normal mouse showing typical hind leg posture when held up by the tail. *B*—Quivering male with priapism. The hind legs had to be restrained from clasping. *C*—Quivering male showing characteristic clasping of hind legs when held up by the tail.

MICE having the condition here called "quivering" appeared in 1953 in the furless stock maintained in the Department of Zoology, at The Ohio State University. The origin of the furless stock of mice is described by Green[1]. During several generations of close inbreeding no mice of the quivering type had previously been observed. Therefore it was assumed that this condition was the result of a mutation. Genetic testing revealed that the quivering condition depends upon an autosomal recessive gene (gene symbol, *qv*) and that *qv* is in linkage group I.

Description

Homozygous quivering mice are characterized by: (1) locomotor instability from as early as 14 days of age; (2) pronounced quivering with increasing age; (3) varying degrees of paralysis of the hind legs and stiffening of the tail from about 30 days onward; (4) locking of the hind legs in a tonic spasm when held up by the tail (Figure 21); (5) priapism in a majority of males, especially several days before death; (6) eye infection; (7) deafness; (8) sterility of males. Quivering females are fertile and nurse their litters. Quivering males have been found, upon dissection, to have viable sperm, but none have sired litters. It may be that the paralysis of the posterior part of the body prevents normal copulation. The life span of quivering mice is short, the majority dying before five months of age.

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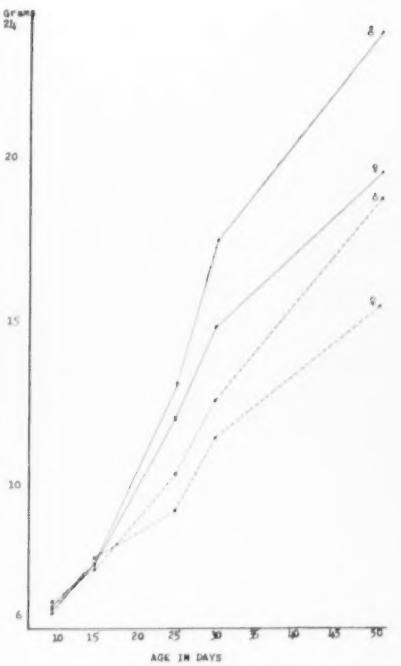
Body Weight

The body weight of normal and quivering mice was compared. Since the body weight of young mice varies greatly with the size of the sibship only litters of nine and 10 offspring were chosen and the animals were weighed on the 10th, 15th, and 25th day after birth. They were weaned on the 25th day. After weaning, the same number of normal and quivering mice of the same sex was chosen from each litter and they were weighed on the 30th and 50th day. Table I shows that there is no difference in body weight up to the 15th day. On the 25th day, however, the weights differ, the quivering mice being lighter than normal by an average of 3.69 grams in males and 1.74 grams in females. This difference increases as the mice grow older, reaching 5.11 grams in males and 3.94 grams in females on the 50th day (Figures 22 and 23). Starting sometime after the 50th day of age, the majority of quivering mice invariably begin to lose weight. The age at which weight loss begins varies considerably. Within two weeks of the beginning of weight loss the hind legs become completely paralyzed. The mice lie on their backs or sides most of the time, unable to take either food or water. Death follows shortly thereafter. The body weight of adult quivering mice at death is approximately 10 grams.



GROWTH CURVES
Figure 22

Growth curves for normal and quivering mice: Solid lines indicate normal; broken lines indicate quivering.

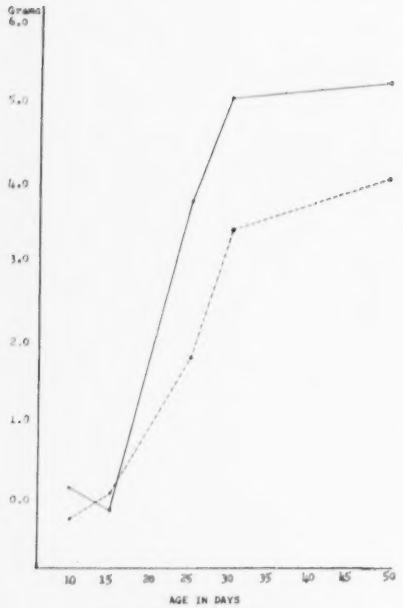
Genetics

Fifteen quivering females were mated with males of the BALB/c strain. These matings produced 20 F_1 litters consisting of 128 normal males and females. In 60 F_2 litters there were 337 normal and 94 quivering mice. These numbers do not differ significantly from the expected 3:1 ratio of normal to quivering if quivering is due to an autosomal recessive gene. Similar matings were made between

TABLE I. Body weight difference between normal and quivering mice in grams*

Sex of Mice	Age in Days	Number of Animals Normal	Number of Animals Quivering	Mean Body Weight Normal	Mean Body Weight Quivering	Difference of Means	t	P
Male	10	12	12	6.18±0.84	6.07±0.89	−0.11	0.275	>.05
	15	12	12	7.56±0.83	7.70±0.93	+0.14	0.350	>.05
	25	12	12	12.87±1.58	9.18±1.33	−3.69	5.426	<.05
	30	12	12	17.46±2.12	12.51±2.48	−4.95	4.304	<.05
	50	10	10	23.62±1.50	18.51±1.79	−5.11	6.231	<.05
Female	10	10	10	6.14±0.98	6.39±1.12	+0.25	0.781	>.05
	15	10	10	7.56±1.01	7.47±1.17	−0.09	0.145	>.05
	25	10	10	11.99±1.59	10.25±1.64	−1.74	1.832	>.05
	30	10	10	14.76±1.96	11.43±2.09	−3.33	2.846	<.05
	50	8	8	19.33±1.23	15.39±1.99	−3.94	4.104	<.05

*From 10 litters of size nine and ten.



WEIGHT DIFFERENCES

Figure 23

Illustrated graphically are the weight differences between normal and quivering mice. Weight of quivering is subtracted from that of normal. Solid line = males. Broken line = females.

six quivering females and males of the C57BL/10 strain. Six F_1 litters consisting of 30 normal males and females were produced. In 65 F_2 litters 399 normal and 126 quivering mice were observed. Again, these numbers do not differ significantly from the expected 3:1 ratio. As a further test F_2 normal mice were mated with F_1 normal mice. Altogether 91 F_2 normal mice, including 34 males and 57 females, chosen randomly, were mated to F_1 mice. If the mutation is due to an autosomal recessive gene one-third of the F_2 normal mice should have been homozygous normal and two-thirds should have been heterozygous carriers of the quivering gene. A test mouse was classified as homozygous normal when it had produced at least 11 normal offspring. This insures an accuracy beyond 95 percent. There were only two cases in which mice were classified as homozygous normal after they each had produced nine normal offspring. This was due to the deaths of the test mice. Thus there were 33 homozygous normal mice and 58 heterozygous carriers. These numbers do not differ significantly from the expected ratio of one homozygous normal to two heterozygous carriers ($\chi^2 = 0.353$, d.f. = 1, $P > .05$).

Linkage

It was found during the experiment that the gene *qv* is linked with *c*, albinism, in linkage group I. The BALB/c males carried *cc* and the quivering females *CC*. Therefore, the matings between BALB/c males and the quivering females were $\left(\frac{c+}{c+} \times \frac{+qv}{+qv}\right)$ type and matings between their F_1's were $\left(\frac{c+}{+qv} \times \frac{c+}{+qv}\right)$ type (repulsion). Fifteen such F_1 matings produced a total of 431 F_2 mice. As indicated in Table III, there was a significant deficiency of the double recessive class, while the three remaining classes contained some excess in comparison with the 9:3:3:1 ratio expected on the basis of independent assortment. A χ^2 for three degrees of freedom calculated for these four classes has the value of 18.76 which is significant at the 5 percent level. In order to find the source of the deviation the χ^2 was subdivided into its three components. The

TABLE II. Results of mating quivering with normal BALB/c and with normal C57BL/10 mice

Mating Sire	Dam	Normal		Quivering		Total
Balb/c × Quivering		65	63	0	0	128
F_1 × F_1		168	169	41	53	431
Observed F_2		337		94		
Expected 3:1		323.2		107.8		
		$\chi^2 = 2.340$ d.f. = 1 P >.05				
C57BL/10 × Quivering		15	15	0	0	30
F_1 × F_1		208	191	53	73	525
Observed F_2		399		126		
Expected 3:1		393.8		131.2		
		$\chi^2 = 0.284$ d.f. = 1 P >.05				

TABLE III. Results of linkage test with albinism and pink eye

Types of Mating								Recombination Value				
$\frac{+\ qv}{+\ qv}$	×	$\frac{+\ qv}{c\ +}$	$\frac{CQv}{+\ +}$	$\frac{cQv}{+\ +}$	$\frac{Cqv}{+\ +}$	$\frac{cqv}{+\ +}$	Total		Segregation for C, c	0.947	1	$>.05$
									Segregation for Qv, qv	2.339	1	$>.05$
$\frac{c\ +}{c\ +}$			244	93	88	6	431	27.6±5.0	Joint Segregation	15.474	1	$<.05$
Expected 9:3:3:1			242.4375	80.8125	80.8125	25.9375			Total	18.760	3	$<.05$
$\frac{+\ +}{+\ +}$	×	$\frac{+\ +}{c\ qv}$	$\frac{CQv}{+\ +}$	$\frac{cQv}{+\ +}$	$\frac{Cqv}{+\ +}$	$\frac{cqv}{+\ +}$			Segregation for C, c	1.452	1	$>.05$
									Segregation for Qv, qv	3.586	1	$>.05$
$\frac{c\ qv}{c\ qv}$			124	22	18	16	180	28.8±4.1	Joint Segregation	13.521	1	$<.05$
Expected 9:3:3:1			101.25	33.75	33.75	11.25			Total	18.559	3	$<.05$
$\frac{+\ +}{+\ +}$	×	$\frac{+\ +}{p\ qv}$	$\frac{PQv}{+\ +}$	$\frac{pQv}{+\ +}$	$\frac{Pqv}{+\ +}$	$\frac{pqv}{+\ +}$			Segregation for P, p	0.349	1	$>.05$
									Segregation for Qv, qv	0.511	1	$>.05$
$\frac{p\ qv}{p\ qv}$			233	20	21	71	345	12.4±1.9	Joint Segregation	180.677	1	$<.05$
Expected 9:3:3:1			194.0625	64.6875	64.6875	21.5625			Total	181.537	3	$<.05$

component of χ^2 due to the deviation of $+$, c segregation from the 3:1 ratio was found by adding the first and third classes together and the second and fourth classes together, taking the difference and basing the calculation on this according to the method of Mather[4]. The χ^2 was found to be 0.947 for one degree of freedom. Similarly, χ^2 for the deviation of the $+$, qv segregation from 3:1 was found to be 2.33 with one degree of freedom. Neither of these values is significant at the 5 percent level. However, the third component, that indicating linkage, is based on the difference between the sums of the first and fourth classes and the second and third classes (Mather[4]). This component of χ^2 was found to be 15.47 for one degree of freedom which is significant at the 5 percent level. Therefore the inference can be made that the genes c and qv are linked.

Matings of $\left(\frac{c\ qv}{c\ qv} \times \frac{++}{++}\right)$ were made, using the C57BL/10 strain as parents. In these matings the F_1 mating type was $\left(\frac{++}{c\ qv} \times \frac{++}{c\ qv}\right)$ (coupling). From six such F_1 matings a total of 180 F_2 mice were obtained. As indicated in Table III, the data again show linkage between c and qv.

In order to determine the sequence of these genes in the first linkage group, mice of genotype $\frac{p\ qv}{p\ qv}$ were produced by appropriate matings. No new strain of mice was necessary for the production of these mice since p was present in the quivering stock. Then matings of $\left(\frac{p\ qv}{p\ qv} \times \frac{++}{++}\right)$ were made, using C57BL/10 strain as $\frac{++}{++}$ parents. In these matings the F_1 mating type was $\left(\frac{++}{p\ qv} \times \frac{++}{p\ qv}\right)$ (coupling). Nine F_1 matings produced 345 F_2 mice. As seen in Table III, there was a significant excess in both the first and the last classes, while the two remaining classes showed a significant deficiency in comparison with the 9:3:3:1 ratio expected on the basis of independent assortment. The analysis of χ^2 tests again indicates a rather close linkage between p and qv. (Table III)

Using the maximum likelihood method described by Mather[4] the recombination values between c and qv and p and qv were computed. Because of the sterility of male quivering mice and because of the poor breeding performance of the females only F_2 data were used for the determination of the values. Out of necessity the recombination frequency in males and fe-

males was assumed to be equal. It was found by this method that the recombination value between *c* and *qv* is 27.6 percent and 28.8 percent in repulsion and coupling respectively. The arithmetic average is 28.2 percent. The recombination value between *p* and *qv* in coupling is 12.4 percent.

Since the recombination value between *p* and *c* is known to be 14.2 (in females, reported by Gruneberg[2]), recombination values between *c* and *qv*, and *p* and *qv* discovered in this experiment indicate that the order of these three genes on the chromosome is *qv*, *p*, *c*. Using these values and the order of genes, the following map was constructed:

:	12.4	*p*	14.2	:
qv		28.2		*c*

Comparison with a Similar Mutation

Among the many neuromuscular mutations, ataxia (gene symbol, *ax*) reported by Lyon[3] resembles quivering most closely. These two mutant types are nearly identical in many respects judging from Lyon's description of ataxia mice. However, they are evidently not the result of the same gene since *ax* assorts independently of the gene *p*, while *qv* is rather closely linked with the same gene. In addition there are some outstanding differences between these mutant types. First, both male and female *axax* mice are reported to be sterile while *qvqv* females are fertile. Second, the difference in body weight between normal and quivering mice seems to be less than that between normal and ataxia mice. Third, some behavioral traits present in ataxia, such as standing on straightened hind legs, are absent in quivering mice, while a trait such as priapism present in quivering male mice is not reported in male ataxia mice.

Summary

A new mutation, quivering (gene symbol, *qv*) was found to depend upon an autosomal recessive gene. The syndrome of quivering mice is described. The gene *qv* is linked with *c* (albinism) and *p* (pink eye) in linkage group I. There are certain similarities between quivering and ataxia mice, but these phenotypes are evidently not the result of the same gene.

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INHERITANCE OF SOME OF THE MORE STRIKING CHARACTERS IN RICE

NELSON E. JODON*

THE genetics of seven new or recently reported characters on which there was no linkage data, and of 12 characters of longer standing, were studied in 10 crosses at the Rice Experiment Station, Crowley, Louisiana. Early Prolific long glume, blackleaf, and neckleaf were mutations in standard varieties occurring in the field; whitehull was present in an introduced variety, yellowhull, long extra lemma, and a dwarf type were first noted in segregating populations. Among the other characters segregating in these crosses, those most notable for their rather bizarre effect included clustered spikelets, liguleless, and the fragile plant character brittle. The chi-square test was used to measure the independence of inheritance (of the paired combinations) among the F_2 segregations in each of the crosses.

The crosses were made in the field using the hot-water method of emasculation[4]. Possibly the long lemma and dwarf characters were mutations due to heat. The heat treatment is applied in the morning prior to the beginning of natural blooming. It stimulates the florets to open immediately, or at least earlier than they normally would, and allows crosses to be made before much pollen is present in the air.

The seed obtained by cross pollination were sprouted on absorbent cotton and the seedling F_1 plants were transplanted to the unflooded field after about 10 days. Classification for F_2 plant color characters was made at maturity, and a single panicle was saved from each plant and used for classification of other characters in the laboratory. The F_2 populations were grown in various years.

The system of symbolization for rice characters proposed by Kadam and Ramiah[15] is followed here. Their symbolization, however, has been criticized by Nagao who proposes another set of symbols in his review of Japanese work on rice genetics[21].

Description and Inheritance of Characters

For brevity, detailed descriptions and complete data on F_2 segregations are given here only for the seven characters tested for linkage for the first time. These and other characters studied in conjunction with them are illustrated in the accompanying photographs, so far as possible.

Segregation of nine of the 12 characters classified in conjunction with the seven "new" ones was probably dependent on a single gene in each of the crosses studied, although poor agreement with the calculated 3:1 ratio was obtained in certain families. Segregation for awns was in a ratio of 13 awnless: 3 awned[6], indicating the interaction of a dominant gene for awns and an awn inhibitor. The segregation of purplehull vs. nonpurplehull apparently was due to more than one gene, but was not in agreement with any ratio, possibly because of fading. In previous reports[1,7,15], the segregation of purple vs. nonpurple pericarp depended on a single gene difference, but in the F_2 of cross 182 in this study a dihybrid ratio was obtained. There were 159 plants having purple pericarp: 103 with nonpurple pericarp. Chi-square for goodness of fit for a calculated 9:7 ratio was 2.096, showing good agreement.

Of the 19 characters studied, eight involved the mature spikelet; long lem-

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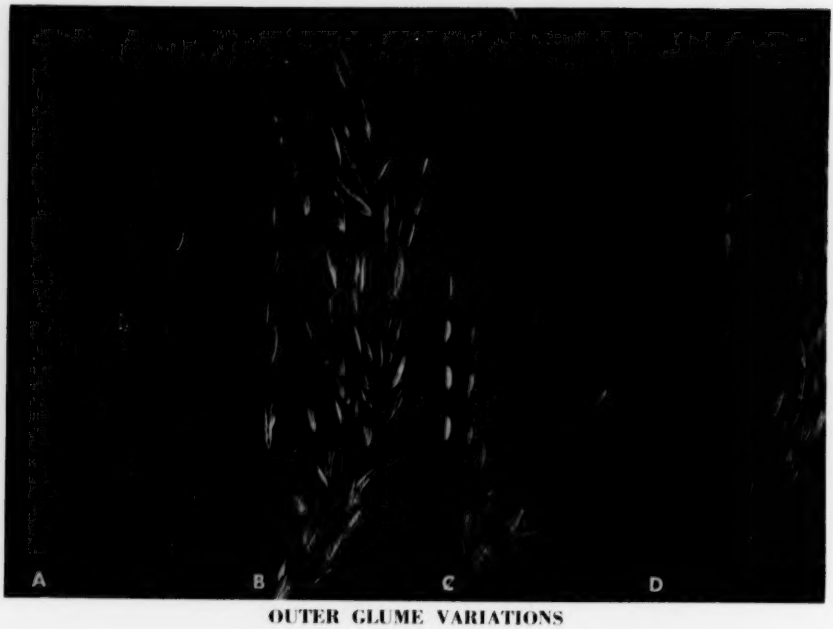


OUTER GLUME VARIATIONS

Figure 24

A—Recessive long glume. *B*—Semi-dominant long glume mutation from Early Prolific. *C*—Heterozygous for Early Prolific long glume. *D*—Ordinary short glume. (The grains in *C* are waxy—see Figure 27. The others have red and/or purple pigmentation in the bran.)

mas, two types of long outer glume[3,11,15], awns[1,2,6,7,11,18], straw vs. goldhull[9,7], whitehull, yellowhull, and waxy (glutinous) endosperm[2,3,7]. Four anthocyanin color characters are also usually distinguishable in the mature spikelet; purple apiculus[2,10,21,22], purplehull[7,10,21], purple pericarp[1,7], and red pericarp[10,21,24]. Clustered describes a clumped arrangement of the spikelets on the panicle branches[5]. Four characters involve the leaves; pubescence, which is also present on the lemma and palea[7,9], blackleaf color, liguleless[11], and neckleaf[12,21]. The two characters remaining, brittle[11] and dwarf, affect the entire plant.

Characters for which linkage tests are reported for the first time are described below.

Early Prolific Long Glume (Gl)

This character (Figure 24*B*) was discovered in 1935 by W. A. Douglas, U.S.D.A. entomologist, who collected the original panicle in a field of Early Prolific. The outer glumes are longer and less uniform through the panicle than in C. I. 4011 and other long glume introductions (Figure 24*A*).

Two crosses, numbered 466 and 684, between the Early Prolific long glume mutation and varieties having ordinary short glumes were grown. The F_2 population was classified in three groups; long glume, intermediate as in the F_1, and short glumes as shown in Table I. The segregation of cross 684 was in agreement with a 1:2:1 ratio of the respective classes, although the number of plants in the long glume class was less than the calculated number. The segregation of cross 466 was in rather poor agreement with a 1:2:1 ratio, due to an excess of short glume plants. The results from cross 684 indicate, however, that segregation was controlled by a single gene lacking dominance. Ramiah *et al*[23], reported a 1:2:1 ratio for a possibly similar long glume character.

Somewhat less clear cut results were obtained from cross 848, a short glume and brittle culm selection × an F_3 Early Prolific long glume selection, and cross 852, Early Prolific long glume × a short glume clustered spikelet selection. The short glume parents were unrelated but similar in having

spikelets very thick in proportion to length. In the other crosses reported here the short glume parents were of intermediate or long grain type, although not extremely slender.

Very few F_2 segregates of either of these two crosses resembled the long glume parent in having the outer glumes longer than the lemma and palea. Nor were the intermediate and short glume types so obviously distinct as in the other crosses. The following numbers were obtained from the classification of cross 848; 12 long glume: 140 intermediate long glume: 427 short glume. In cross 852 the numbers similarly were 6:143:342.

Possibly measurements of glume length would have shown a 1:2:1 distribution, but seemingly, in the short broad spikelet the outer glumes were telescoped to a degree which made it impossible to make an accurate visual separation of the homozygous long glume, heterozygous intermediate glume, and homozygous short glume plants. The segregation for shape of spikelet was not studied in these crosses, but it was noted that the thick short type predominated so that in a large proportion of each population the expression of the long glume character may have been influenced by a modifying effect exerted by genes controlling spikelet shape.

Only 36 and 48 of the apparently short glume plants from the respective crosses were used for F_3 tests of homozygosity. Respectively, 14 and 18 of these were noted to be segregating for the long glume character, showing that nearly one-half of the genetically intermediate long glume F_2 plants had been placed in the short glume class. This indicates the presence of modifier genes reducing glume length, and it is possible that these may be the same as or closely linked with those controlling shape of spikelet.

The Early Prolific long-glume strain was also crossed with C. I. 4011, a variety having glumes about two-thirds as long as the lemma and palea. The C. I. 4011 long glume was known to be completely recessive to the ordinary short glume type[1]. Kadam *et al*[10] reported a similar recessive long glume mutation. The F_1 had outer glumes nearly one-half the length of the lemma and palea, similar to the F_1 of crosses with short glume varieties. An F_2 population of 262 plants from two families was grown, and results of classification are shown in Table II.

Four phenotypic classes were recovered in the F_2; (1) Early Prolific long glume type, (2) an intermediate type similar to the F_1, (3) the ordinary but non-parental short glume type, and (4) C. I. 4011 long glume type. The distribution of F_2 plants among these four classes was in a 4:8:3:1 ratio. The chi-square test for goodness of fit indicated good agreement of observed with calculated numbers.

These results indicate segregation for two independent pairs of genes both affecting length of glume, but having different expression. In addition the long glume gene from Early Prolific was epistatic to the one from C. I. 4011. The Early Prolific long glume gene also showed lack of dominance, which interpretation is supported by the intermediate condition of the F_1. The short glume plants resulted from new combinations of the two genes, the Early Prolific long glume strain contributing the dominant short glume allele of the C. I. 4011 long glume gene.

These results tend to bear out the conclusion drawn from cross 684 that the long glume condition of the Early Prolific mutant is due to a single gene showing lack of dominance. However, this was not demonstrated in crosses involving short thick grain types, probably because of the influence of genes for spikelet shape. The symbol *Gl* is suggested to represent this gene.

TABLE II. F_2 segregation of outer glumes types in cross 182, C. I. No. 4011 recessive long glume (g) × Early Prolific long glume mutation (*Gl*)

F_2 Phenotypes	Ratio	Numbers Obs.	Calc.
Early Prolific type (*Gl Gl*)	4	129	131
Intermediate type (*Gl gl*)	8	67	65.5
Ordinary Short type (*gl G*)	3	48	49.1
Recessive Long type (*gl g*)	1	18	16.4
Total =		262	262.0
χ^2 =			0.246
P =			.98 - .95

TABLE I. F_2 segregation of Early Prolific long glume mutation (*Gl*) in cross 466, Recessive Selection (*gl*) × Early Prolific Long Glume (*Gl*) and in cross 684, Early Prolific Long Glume (*Gl*) × Neckleaf Caloro (*gl*)

F_2 Phenotypes	Ratio	Numbers Cross 466 Obs.	Calc.	684 Obs.	Calc.
Long (*Gl Gl*)	1	28	40.5	24	28.25
Intermediate (*Gl gl*)	2	83	81.0	58	56.50
Short (*gl gl*)	1	51	40.5	31	28.25
Total =		162	162	113	113
χ^2 =			6.629		0.947
P =			.05 - .02		.70 - .50

LONG EXTRA GLUME
Figure 25
A shows the relationship between flowering and outer glumes. *B* shows other floret abnormalities.

Long Lemma (lmx)

This character (Figure 25*A* and *B*) appeared in the F_2 of cross 852, but was not noted in either parent. It did not appear in any other cross involving Early Prolific long glume, so it is unlikely that it is an expression of the gene for that character. Only occasional spikelets exhibit the extra length in the lemma or have an extra glume between the lemma and the outer glume.

Long lemma resembles one of the floret abnormalities described by Kuang *et al.*[17] under the general term "polyhusk." It resembles most closely the condition shown in their Figure 4. They obtained 3:1 ratios for normal vs. various "polyhusk" types.

The F_2 segregated in a ratio of 382 normal: 109 long lemma. Chi-square was 2.054, indicating good agreement with the calculated 3:1 ratio. F_3 progeny of F_2 segregates show that the abnormality bred true. It was concluded that the character is due to a single recessive gene, and the symbol *lmx* is suggested to represent it.

Hull Colors, Whitehull (HwHg) and Yellowhull (Hwhg)

Whitehull, in contrast to strawhull color, appears dull chalky white, (Figure 26*B* and *D*). Possibly it is due to the absence of some pigment occurring in strawhull color rice varieties, ordinarily considered colorless. It appears in strains both with and without purple apiculus, indicating that it has no relation to anthocyanin formation.

The whitehull strain used as a parent in crosses 844 and 846 was a selection from the cross C. I. 6121-3 × C. I. 3212, and was made available through the courtesy of Mr. H. M. Beachell, Rice-Pasture Experiment Station, Beaumont, Texas. Whitehull was a characteristic of the introduction C. I. 3212.

The inheritance of whitehull was studied in two families of cross 844, between whitehull and a strawhull selection. The F_1 was whitehull and the F_2 segregated 856 whitehull: 263 strawhull. Chi-square for goodness of fit for a 3:1 ratio was 0.427, indicating good agreement. It was concluded that the whitehull character is due to a single dominant gene, which was assigned the symbol *Hw*.

In addition, whitehull was studied in cross 846, between whitehull and goldhull. Goldhull is the recessive allele of strawhull. Four F_2 phenotypes appeared as shown in Table III including two non-parental types, *yellowhull* and *strawhull*, in addition to the whitehull and goldhull parental classes.

These results indicate that the dominant gene for whitehull is not allelic to the goldhull gene. Yellowhull is a new character produced by the diluting effect of whitehull on

HULL COLORS
Figure 26

A—Purple hull; *B*—Whitehull—epistatic to strawhull; *C*—Ordinary strawhull; *D*—Light yellow hull (goldhull modified by whitehull); *E*—Ordinary goldhull, recessive to strawhull.

the goldhull color pigment, so that all plants with *HwHw* or *Hwhw* and homozygous goldhull genes have yellowhull color. Strawhull appears in the absence of the whitehull, which is epistatic to it. Goldhull appears when both pairs of genes are completely recessive. The expected ratio on this basis is 9 whitehull: 3 yellowhull: 3 strawhull: 1 goldhull. Chi-square for goodness of fit for this ratio was 3.579, indicating good agreement.

Yellowhull is not easily distinguished from strawhull, and classification should be made promptly at full maturity because strawhull color grains may take on a yellowish tinge after being in storage for some time.

Blackleaf (lk)

Blackleaf (Figure 28*B* and *C*) is a discoloration that appears first on the leaves and sheaths as dark brown spots resembling fungus lesions. It spreads and by maturity extends even into the panicle branches, giving the entire plant a blackened appearance. It originated as a single plant found by the writer in 1945 in a field of Magnolia being increased at the Rice Experiment Station.

ENDOSPERM TYPES
Figure 27

A—Ordinary clear endosperm, staining blue with iodine. *B*—Waxy (glutinous) opaque white endosperm, staining red.

Jones[12] reported two mutations in Caloro similar to blackleaf which he termed physiologic diseases. One segregated in a ratio of 1 normal: 2 intermediate: 1 diseased, and the other segregated 3 normal: 1 diseased. The affected plants, especially of the first type, were distinctly shorter than the normal. When these mutations were grown at Crowley they showed less discoloration than the Magnolia mutation. No tests have been made to

TABLE III. F_2 segregation of hull colors in cross 846, Goldhull (*hg*) × Whitehull (*Hw*)

F_2 Phenotypes	Genotypes	Ratio	Numbers Obs.	Calc.
Whitehull	*Hw–Hg–*	9	632	668.25
Yellowhull	*Hw–hghg*	3	248	222.75
Strawhull	*hwhw Hg–*	3	234	222.75
Goldhull	*hwhw hghg*	1	74	74.25
	Total =		1188	1188.00
	χ^2 =			3.579
	P =			.50 - .30

THREE STRIKING CHARACTERS

Figure 28

A—Clustered florets; *B*—Blackleaf mutation in Magnolia. *C*—Liguleless.

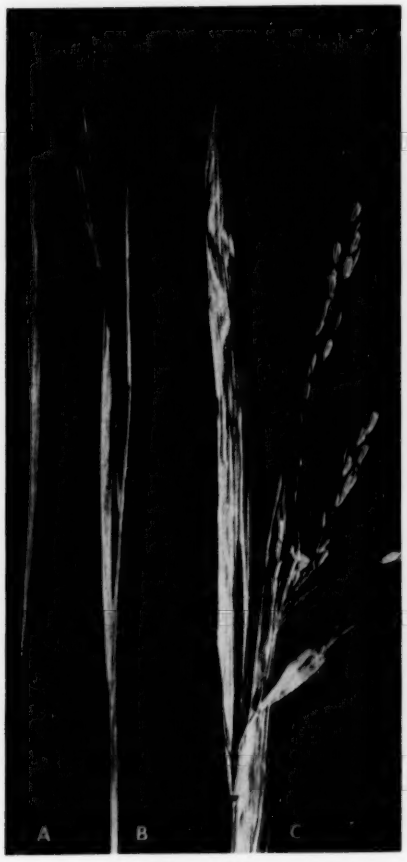
determine if the mutations are due to allelic genes.

The inheritance of the Magnolia blackleaf character was studied in the F_2 of crosses 839, 840, and 842. In each of these crosses the segregation was in good agreement with a ratio of 3 normal: 1 blackleaf. The combined results from the three crosses were in a ratio of 1897 normal: 600 blackleaf. Chi-square for goodness of fit for a 3:1 ratio was 1.813, indicating that segregation was controlled by a single gene. The symbol *lk* was assigned.

Both growth and grain yields of blackleaf plants are reduced to a marked degree. Thirteen normal F_3 rows averaged 175 grams per row, whereas the same number of blackleaf rows averaged only 114 grams per row.

Neckleaf (nk)

Neckleaf (Figure 29*A-C*) is a spathe-like bract arising at the basal node of the panicle. There is no distinct division between sheath and leafblade. Neckleaf was found as a mutation in the Caloro variety at Biggs, California, by the late J. W. Jones, who reported



NECKLEAF

Figure 29

A—Panicle partially enclosed in sheath arising at basal node of panicle. *B*—Panicle completely enclosed. *C*—Additional bracts at base of some of the branches of the panicle.

DWARFING

Figure 30

A—Panicle from normal size plant. *B*—Dwarf, note crinkling of basal part of flag leaf. *C*—Dwarf plant with crinkling, but having spikelets of nearly normal size.

that it differs from normal by a single recessive gene[12]. Mr. Jones kindly supplied seed of this spectacular mutant. Nagao[21] described a similar single recessive gene mutation found by a Japanese worker. The two mutations have not been tested to determine if they are allelic.

F_2 data were obtained from crosses 684 and 848; each segregated in a ratio of 3 normal: 1 neckleaf. The combined populations of the two crosses segregated in a ratio of 513 normal: 179 neckleaf. Chi-square for goodness of fit for a 3:1 ratio was 0.277 indicating good agreement.

In some true-breeding lines neckleaf is longer than the panicle and essentially encloses it. Some seed are formed, although florets on panicles thus enclosed apparently do not open. Enclosed panicles are subject to attack by fungi which also reduces seed set.

Some neckleaf segregates developed additional bracts at the bases of the branches of the panicle, a modification which has not been mentioned in prior reports. The occurrence of extra bracts appears to be genetic. Both crosses reported here were also segregating for the Early Prolific type long glume, but

TABLE IV. Chi-square (for independence) from 31 combined ratios of Early Prolific long glume, long lemma, neckleaf, dwarf, and 10 other characters segregating in the F_2 of five rice crosses. The genetic constitution of the parents was as follows:

Cross No. 182 C. I. 4011 (*gl g Prp Prp*$_s$ *Pr wx*) × Long Glume Early Prolific (*Gl G prp*$_s$ *prp*$_s$ *pr Wx*)
Cross No. 466 Selection (*gl lh hg wx*) × Long Glume Early Prolific (*Gl Lh Hg Wx*)
Cross No. 684 Long Glume Early Prolific (*Gl lm Nk*) × Neckleaf Caloro (*gl lm nk*)
Cross No. 848 Selection (*gl*^{a} *lm Nk bc Ap*) × Selection (*Gl LN nk Bc ap*)
Cross No. 852 Long Glume Early Prolific (*Gl*^{a} *Lmx*^{a} *D*$_e$^{a} *scl ap*) × Selection (*gl lmx*^{a} *d*$_e$^{a} *Scl Ap*)

	Gl	*Lmx*	*Ldn*	*hg*	*Scl*	*wx*	*Ap*$_n$	*Prp*	*nk*
Long glume vs. short	*Gl*								
Normal vs. long lemma	*lmx*	97.226							
Short vs. long glume (C. I. 4011)	*g*	0.246**							
Awnless vs. awned (13.3)	*Ldn*	0.085							
Straw vs. gold hull color	*hg*	3.391							
Clustered vs. normal spikelets	*Scl*	0.125	30.475						
Starchy vs. waxy (glutinous) endosperm	*wx*	2.070		0.181					
Purple vs. colorless apiculus	*Ap*$_n$	0.150	5.070	1.564	11.640				
Purple vs. non purple pericarp (9.7)	*Prp*	3.002				0.052			
Red vs. colorless pericarp	*Pr*	3.812				0.357		1.826	
Pubescent vs. glabrous	*lh*	0.085		1.261		3.804			
Normal vs. neckleaf	*nk*	0.0001	0.935				0.224		
Normal vs. brittleculm	*bc*	0.0003	5.192				3.855		0.155
Normal vs. dwarf	*d*$_e$	0.739	5.659		2.599		0.053		

*Segregation in poor agreement with expected. **Goodness of fit, 4:8:3:1 ratio, from Table II.

it is not known if this gene influenced the expression of the neckleaf character.

Dwarf (d$_e$*)*

This dwarf (Figure 30*B* and *C*) appeared in the F_2 of cross 852, as did also the long lemma character. It is similar to the thickset dwarf mutation[8,13] but is somewhat less stout and perhaps more variable in size. The F_2 segregated in a ratio of 393 normal: 98 dwarf. These numbers suggest a segregation of 3 normal: 1 dwarf, but chi-square for goodness of fit with this ratio was 6.654 due to the deficiency in the dwarf class. It is possible, however, that this deficiency is due to differential emergence and the loss of dwarf plants by drowning out at first flooding.

Linkage Tests

The linkage between purple apiculus and glutinous (*Ap-wx*) was the second reported in rice and is credited to Takahashi[25]. Yamaguchi[26] referred to it as the second group, but subsequently it has been referred to uniformly as linkage group I [2,7,16,28,19,21]. The data available to 1948 were summarized by Jodon[7], and in 1951 the results of Japanese linkage studies were made available in English by Nagao[21]. Nagao's first group (*gl* linkage group) corresponds to Jodon's group I, his fourth (*Sp* linkage group) to group II, his third (*lng* group) to group IV, and his second (*Pl* group) to group V.

Six genes for the seven characters described above, Early Prolific long glume, long extra lemma, whitehull, yellowhull, neckleaf, blackleaf, and dwarf were tested for linkage in certain combinations with 11 marker and other genes in 10 crosses. Neckleaf and two physiologic disease characters similar to blackleaf have been described in previous publications[12,21], but no linkage tests were reported.

The 10 crosses reported herein afforded only 63 paired combinations of F_2 segregations out of the 153 combined ratios possible among 18 characters. The chi-square for independence method was used to test for association, and the resulting values are summarized in Tables IV, V, and VI. Combined ratios which resulted in chi-square greater than 3.841 ($P = 0.05$), indicating that segregation was not independent, are shown in Table VII.

Table IV summarizes results from five Early Prolific long glume crosses, from which 31 combined ratios of paired segregations were available for chi-square tests. Segregation for the previously untested characters, long extra lemma, dwarf, and neckleaf also occurred in these crosses.

Combined ratios of 13 paired segregations involving the Early Prolific long glume character were obtained, including combinations with group I marker genes *Ap, wx,* and *Scl;* group II marker *hg,* group IV marker *g;* group V marker *lg;* and group VII marker *lh*[7].

The chi-square tests indicate independent assortment of the Early Prolific long glume gene with each of these marker genes and also with genes for neckleaf, dwarf, awned, brittle, and red and purple pericarp. An association with long extra lemma was indicated (refer to Table VII), but probably this was due to an excess in the *Lmx gl* class brought about by lack of clear-cut segregation in cross 852.

Association in cross 852 of long lemma with clustered and with apiculus color (Ap_a) in group I, and also with dwarf, was indicated by the chi-square tests. A part of the association between *lmx* and *scl* appeared to be due to a deficiency in the double recessive class, but possibly it was due in part to linkage in the repulsion phase. It was concluded, however, that long lemma probably is not linked in group I. Similarly, deficiency in the double recessive class may account for the apparent association of *lmx* with Ap_a, and with dwarf. There was no indication of association of dwarf with any other character.

The chi-square test of the combined segregation of brittle and awned showed some indication of association between these two characters, but this also may have been due to the deficiency in the double recessive class.

The only combination between marker genes obtained in these crosses was that between apiculus color and clustered. As shown in Table VII, crossing-over was 39 percent which agrees well with previous results[7].

No other association was indicated among the 14 characters segregating in the five Early Prolific long glume crosses, but only 31 of the 91 possible combined ratios were available for testing.

Table V summarizes results from two whitehull crosses, from which 24 combined ratios of paired segregations were available for chi-square tests. There were eight combinations of paired segregations involving the whitehull character, including combinations with group I marker genes Ap_a and *wx*, group II markers Ap_b and *hg*, group V marker *lg*, and group VII marker *lh*, and with one other character, brittle.

The indicated close association between pubescence and brittle in cross 844 apparently was a "genetic joker" rather than linkage. It appears that the plant hairs on brittle plants rubbed off very easily, resulting in their misclassification as glabrous.

Group I marker genes Ap_a and *wx* were found to be associated in cross 844. Crossing-over was about 31.5 percent which is greater than the usual value[7]. Liguleless was independent of *wx*, but association with Ap_a was indicated. It is unlikely, however, that linkage was involved because the Ap_a *lg* class

TABLE V. Chi-square (for independence) from 24 combined ratios of whitehull and eight other characters segregating in the F_2 of two rice crosses. The genetic constitution of the parents was as follows:
Cross No. 844 "Whitehull" (*G lh* Ap$_a$* wx Hw Lg* Bc) × Selection (*g Lh ap$_a$ Wx hw lg bc*)**
Cross No. 846 "Whitehull" (*Hg* Ap$_a$* Hw*) × Selection (*hg ap$_a$ hw*)

		Hw	*Hg*	*g*	*wx*	*Ap$_a$*	*lh*	*lg*
Whitehull vs. non-whitehull	*Hu*							
Strawhull vs. goldhull	*h*	3.579**						
Short vs. long glume	*g*	3.846						
Starchy vs. waxy (glutinous) endosperm	*wx*	2.187		0.004				
Purple vs. colorless apiculus	Ap_a	1.667		2.927	52.622			
Purple vs. colorless apiculus	Ap_b	57.859	77.275					
Pubescent vs. glabrous	*lh*	1.885		3.120	2.564	10.747		
Normal vs. liguleless	*lg*	234.005		0.849	0.994	10.599	3.421	
Normal vs. brittle culm	*bc*	0.505		0.291	0.065	6.325	68.009	0.612

* Segregation in poor agreement with expected.
**Goodness of fit, 9:3:3:1 ratio, see Table III.

TABLE VI. Chi-square (for independence) from 15 combined ratios of blackleaf and five other characters segregating in the F_2 of three rice crosses. The genetic constitution of the parents was as follows:
Cross No. 839 Selection (*lh hg Ap* wx* Lk hp?*) × "Blackleaf" Magnolia (*Lh Hg ap Hp? Wx lk*)
Cross No. 840 "Blackleaf" Magnolia (*ap lk*) × Selection (*Ap Lk*)
Cross No. 842 "Blackleaf" Magnolia (*Lh lk*) × Selection (*lh Lk*)

		lk	*lh*	*hg*	*wx*	*Ap$_a$*
Normal vs. blackleaf	*lk*					
Pubescent vs. glabrous	*lh*	1.84				
Straw hull vs. goldhull	*Hg*	3.454	0.117			
Ordinary vs. waxy (glutinous) endosperm	*wx*	0.050	4.140	6.051		
Purple vs. colorless apiculus	Ap_a	0.001	0.381	0.009	54.957	
Purple vs. colorless hull (9:7)	*Hp*	3.174	0.936	1.437	32.668	15.034**

* Segregation in poor agreement with expected.
**Goodness of fit, 9:3:4 ratio.

was lower and the other classes higher than was calculated. There was no other indication of linkage involving liguleless.

Association between apiculus color (Ap_a) and pubescence was indicated in cross 844, but apparently this was due to an excess in the double recessive class and a deficiency in the double dominant class. Previous results[7] indicate independent inheritance of these characters.

Association was also indicated between apiculus color (Ap_a)and brittle. The *Ap-bc* class was lower and the other classes higher than the expected, indicating that linkage was not the explanation.

Group II marker genes Ap_b and *hg* were found to be associated in cross 846. Crossing-over was 34 percent in the coupling phase which agrees fairly closely with previous results[7].

Association was indicated between whitehull and Ap_b in cross 846, and crossing-over was 36 percent in the coupling phase. Whitehull was also found to be linked with group V marker gene *lg* in the coupling phase in cross 844 with 8 percent crossing-over. These results suggest that groups II and V are actually one, but in that event *Hw* should show association with group II marker gene *hg* in cross 846, unless crossing-over is close to 50 percent. The chi-square test indicated independent inheritance of *Hw* and *hg*, and the

TABLE VII. Combined ratios for which chi-square indicated possible association between characters

Linkage Group	Cross	Combined Ratios				Total Number	χ^2	Crossover Percent
		Hp Wx	*Hp wx*	*hp Wx*	*hp wx*			
I	839	308	125	373	65	871	32.668	22.0
		Ap_a *Wx*	Ap_a *wx*	ap_a *wx*	ap_a *wx*			
I	839	443	176	238	14	871	54.957	25.0
	844	551	273	270	35	1129	52.622	31.5
		Ap_a *Scl*	Ap_a *scl*	ap_a *Scl*	ap_a *scl*			
I	852	308	77	68	38	491	11.640	39.0
		Ap_a *Lh*	Ap_a *lh*	ap_a *Lh*	ap_a *lh*			
I	844	526	298	162	143	1129	10.747	*
		Ap_a *Bc*	Ap_a *bc*	ap_a *BC*	ap_a *bc*			
I	844	679	145	231	74	1129	6.325	*
	848	348	86	105	40	579	3.855	*
		Ap_a *Lg*	Ap_a *lg*	ap_a *Lg*	ap_a *lg*			
I	844	675	149	223	82	1129	10.599	*
		Hg Wx	*Hg wx*	*hg wx*	*hg wx*			
I	839	512	126	169	64	871	6.051	*
		Lh Wx	*Lh wx*	*lh Wx*	*lh wx*			
I	839	507	155	174	35	871	4.140	*
		Lmx Scl	*Lmx Scl*	*lmx Scl*	*lmx scl*			
I	852	271	111	105	4	491	30.475	*
		Lmx Ap_a	*Lmx* ap_a	*lmx* Ap_a	*lmx* ap_a			
I	852	291	91	94	15	491	5.070	*
		Lmx D_a	*Lmx* d_a	*lmx* D_a	*lmx dx*			
(?)	852	297	85	96	13	491	5.659	*
		Lmx Gl	*Lmx gl*	*lmx Gl*	*lmx gl*			
(?)	852	70	312	73	36	491	97.226	*
		Ap_b *Hg*	Ap_b *hg*	ap_b *Hg*	ap_b *hg*			
II	846	677	168	189	154	1188	77.275	34.0
		Ap_b *Hw*	Ap_b *hw*	ap_b *Hw*	ap_b *hw*			
II	846	678	167	202	141	1188	57.859	36.0
		Hw Hg	*Hw Hg*	*hw Hg*	*hw hg*			
II	846	632	248	234	74	1188	1.994	53.0
		Hw Lg	*Hw lg*	*hw Lg*	*hw lg*			
V	844	768	88	130	143	1129	234.005	8.0
		Lh Bc	*Lh bc*	*lh Bc*	*lh bc*			
VII	844	608	80	302	139	1129	68.009	*
		IAn Bc	*IAn bc*	*iAn Bc*	*iAn bc*			
VIII	848	354	110	99	16	579	5.192	*

*Association indicated by Chi-square not due to crossing-over. See text.

calculated crossing-over was 53 percent, coupling phase. It is unfortunate that the four characters were not all segregating in the same cross. However, the genes appear to be arranged in the order:

$$\frac{hg\text{-}34\text{-}Ap_b\text{-}?\text{-}lg\text{-}8\text{-}Hw}{\begin{matrix}36\\53\end{matrix}}$$

Liguleless has been reported by Japanese workers[20, 21] to be linked with a gene for purple leafblade color (Nagao's *Pl*[21]) with about 22 percent crossing-over, and with a hull color gene (*"Rp"*) with 26 to 32 percent crossing-over (group V). The 36 percent crossing-over between *Hw* and Ap_b suggests that *"Rp"* and *"Ap"* may be at the same locus, or closely linked.

In the previous report[7] it was assumed that the apiculus color gene (Ap_b) which is linked with the goldhull color gene (*hg*) is the complementary gene that acts with the basic anthocyanin color gene (Ap_a) to produce colored apiculus. Nagao[21] designated the basic gene as *C* and the complementary activating gene as *Sp*. The latter is reported to be linked with a gene for purple node with about 17 percent crossing-over (*Sp* group) and was distinguished from the gene *"Rp"* linked with liguleless (*Pl* group). In these respects our interpretation that groups II and V may be one and the same does not coincide with Nagao's data.

Table VI summarizes results from three Magnolia blackleaf crosses. Six characters were segregating in cross 839, making it possible to test all of the 15 possible combinations of ratios.

No association was found of Magnolia blackleaf with any other character.

Association of group I genes Ap_a and *wx* was indicated, crossing-over being 25 percent. Purplehull was also found to be associated with *wx*, with 22 percent crossing-over. The segregation of purplehull: purple apiculus: colorless glumes was in poor agreement with a 9:3:4 ratio due to a large excess of plants in the non-purplehull classes resulting presumably from fading. It was concluded that purplehull segregation was independent on two complementary genes, one of which is Ap_a (Nagao's *C*).

Blackleaf segregated independently of group I marker genes Ap_a and *wx*, group II marker *hg*, group VII marker *lh*, and also of purplehull (*Hp*).

Chi-square was greater than 3.841 for the combined ratios of waxy and pubescent, and also for waxy with goldhull. The data indicated weak coupling phase linkage, but the cross was made in the repulsion phase. A deficient number of waxy plants probably was responsible for these results, and both goldhull and pubescent have been reported previously to be independent of waxy[7].

No other associations were indicated among the characters segregating in these crosses. Further crosses are needed to determine the linkage relationship of Early Prolific long glume, blackleaf, neckleaf, long extra lemma, and dwarf. The first three of these characters are especially suitable for linkage studies because usually they are easy to classify.

Summary

The genetics of 19 characters of rice, including six genes tested for linkage associations for the first time, were studied in the F_2 populations of 10 crosses. Early Prolific long glume (*Gl*), blackleaf (*lk*), and neckleaf (*nk*) occurred as mutations in standard varieties. Whitehull (*Hw*) was present in an introduction. Yellowhull resulted from the interaction of *Hw* and goldhull. Long extra lemma (*lmx*) and a dwarf type (d_a) appeared in the F_2 generation of one of the crosses.

Long glume (*Gl*) appeared to be controlled by a single gene lacking dominance, although in two crosses a 1:2:1 ratio was not obtained probably because of modification by genes for short broad spikelets. Whitehull (*Hw*) was epistatic to strawhull (*Hg*), but only partially obscures goldhull (*hg*), resulting in yellowhull (*Hw-hghg*). Long extra lemmas (*lmx*), blackleaf (*lk*), neckleaf (*nk*), and dwarf (d_a) apparently are simple recessives. The other characters segregating in the crosses, including markers, were dependent on single genes except for awned, apiculus color, and purple pericarp color.

Sixty-three combinations of ratios were afforded by the ten crosses, and were tested for associated inheritance by the chi-square for independence method. Certain combinations with marker genes for linkage groups I, II, IV, V and VII were included. Whitehull (*Hw*) was linked with liguleless in group V with 8 percent crossing-over, and also appeared to be linked with a gene for apiculus color (Ap_b) in group II with 36 percent crossing-over, indicating that the genes previously assigned to groups II and V actually are located in the same chromosome. No definite indication of linkage was found for any of the five other genes tested. Additional data were obtained on the linkage of Ap_a-*wx* and Ap_a- *Scl* in group I, and on Ap_b-*hg* in group II.

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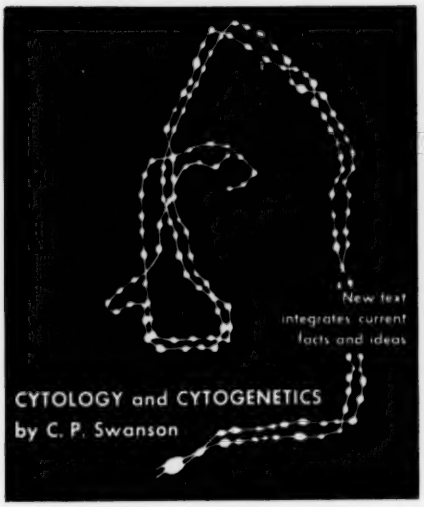

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